ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ASSAY AND DISSOLUTION FOR NISOLDIPINE ER TABLETS BY RP-HPLC

Dissertation work submitted to
The TamilNadu Dr. M.G.R. Medical University, Chennai
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MASTER OF PHARMACY

IN

PHARMACEUTICAL ANALYSIS

Submitted by

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MAY 2012

CERTIFICATE

This is to certify that the dissertation work entitled "**ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ASSAY AND DISSOLUTION FOR NISOLDIPINE ER TABLETS BY RP-HPLC**" is a bonafide work of **Mr.SURESHKUMAR.R** carried out in ORCHID HEALTHCARE,CHENNAI under my guidance and under the supervision of Dr.A.RAJASEKHARA REDDY and has completed to my fullest satisfaction for partial fulfillment of the award of degree of **Master of Pharmacy in Pharmaceutical Analysis**, R V S college of Pharmaceutical Sciences, Sulur, Coimbatore, which is affiliated to The Tamilnadu Dr.M.G.R Medical University, Chennai. It is to certify that the part or whole of the work has not been submitted either to this university or any other university. This work is original and confidential.

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INTERNAL EXAMINER

Place:

Date:

EXTERNAL EXAMINER

Place:

Date:

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ABBREVIATIONS

ACN	:	Acetonitrile
g	:	gram
HPLC	:	High performance liquid chromatography
pH	:	Hydrogen ion concentration
ICH	:	International conference on harmonization
mg	:	Milligram
ml	:	Milliliter
µg	:	Microgram
µg/ml	:	Microgram per milliliter
µg	:	Microgram
nm	:	Nanometer
PA	:	Purity Angle
PDA	:	Photo diode array
TH	:	Purity threshold
RSD	:	Relative standard deviation
k	:	Retention time
SD	:	Standard deviation
UV	:	Ultra violet
V/v	:	Volume by Volume

CHAPTER	TITLE	PAGE

CONTENTS

1. GENERAL INTRODUCTION

Pharmaceutical Analysis plays a major role today, and it can be considered as an interdisciplinary subject. Pharmaceutical Analysis derives its principles from various branches like chemistry, physics, and microbiology etc., Pharmaceutical Analytical techniques are applied mainly in two areas, viz quantitative analysis and qualitative analysis, although there are several other applications.

Drugs and pharmaceuticals are chemicals or like substances, which are of organic, inorganic or other origin. Whatever may be the origin, we use some property of the medicinal agent to measure them quantitatively or qualitatively. Pharmaceutical Analytical techniques, which are being used, can be categorized as follows.

Spectral Methods[1, 2]

We use light absorption (or) emission characteristics of drugs.

E.g. UV spectroscopy, IR spectroscopy, NMR spectroscopy, ESR spectroscopy, mass spectrometry, flourimetry.

Chromatographic Methods

We use affinity or partition coefficient differences between drugs.

E.g. Thin Layer Chromatography, High Performance Liquid Chromatography, Gas chromatography, Paper Chromatography.

Electro Analytical Techniques

Based on the electrochemical property of drugs.

e.g., Potentiometer, Conductometry, Polarography, Amperometry, Paper Electrophoresis.

Radio Active Methods

It involves measurement of the intensity of the radiation from a naturally radioactive substance or an induced radioactive substance arising from exposure of the sample to a neutron source.

e.g., Radio Immuno Assay

Physical Methods

We measure some physical characteristics of drugs

e.g. Differential Thermal Analysis, Differential Scaning Calorimetry, Thermo Mechanical Analysis, Thermo Gravimetric Analysis.

Titrimetric Methods

e.g. Non- aqueous titrations, redox titrations, diazotization titrations and complexometric titrations.

X-Ray Methods

When high speed electrons collide with a solid target, X-rays are produced. From the remittent x-ray emission, it is possible to identify certain emission peaks, which are characteristic of elements contained in the target. The wavelength of the peaks can be related to the atomic numbers of the elements producing them

In recent years, several analytical techniques have been evolved that combine two or more methods into one called "hyphenated" technique eg: GC/MS, LC/MS etc. The complete analysis of a substance consists of four main steps.

1. Sample preparation / Sampling
2. Dissolution of the sample, conversion of the analyte into a form suitable for measurement.
3. Measurement
4. Calculation and interpretation of the measurement

Factors affecting the choice of analytical methods

Possible interference from components of the material other than those of interest.

- The concentration range, which needs to be investigated
- The accuracy required.
- The facilities available.
- The time required for complete analysis.
- Problem arising from the nature of the material

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

One of the early problems with liquid state chromatography was the slow rate at which analysis took place. Early methods use gravity feed, and it was not uncommon for an analysis to take several days to complete. This led not only to great delay but also the excessive time on the column and thus inevitably led to loss of resolution by diffusion and soon. Consequently for a number of year's liquid chromatography was not widely used as a means of separating organic compounds. This problem was largely overcome

by the advent of High Performance liquid Chromatography (HPLC). In this system the pressure is applied to the column forcing the mobile phase through at much higher rate.
The pressure is applied using pumping system. The action of the pump is critical, since it must not pulsate and mix up the sample being separated in the solvent, causing it to lose resolution. Development of pumps have proceeded quite quickly over the last several years, and now it is possible to achieve good separation under the condition required for HPLC. All of the factors affecting separation in liquid chromatography apply to HPLC. The factors affecting plate height, the sample distribution between the stationary and mobile phase and the selection of stationary and mobile phase still pertain even under the conditions of HPLC. The principal advantage of the system is the speed at which separations take place.

Principle of separation in HPLC [3, 4, 5]

The principle of separation can be either adsorption or partition.

Types of HPLC techniques

Based on modes of separation

1. Reversed phase chromatography
2. Normal phase chromatography

Based on principle of separation

1. Adsorption chromatography
2. Partition chromatography
3. Ion exchange chromatography
4. Ion pair chromatography
5. Size exclusion or Gel permeation chromatography
6. Affinity chromatography
7. Chiral phase chromatography

Based on elution technique

1. Isocratic Separation
2. Gradient Separation

Based on the scale of operation

1. Analytical HPLC

2. Preparative HPLC

Based on the type of analysis

1. Qualitative Analysis
2. Quantitative Analysis

Advantages of HPLC

- Efficient, highly selective and widely applicable
- Only small sample is required.
- Ordinarily nondestructive to sample.
- Readily adaptable to Quantitative analysis
- Simple and inexpensive equipment compared to GC.
- Can accommodate nonvolatile and thermally unstable samples.
- Generally applicable to inorganic ions.

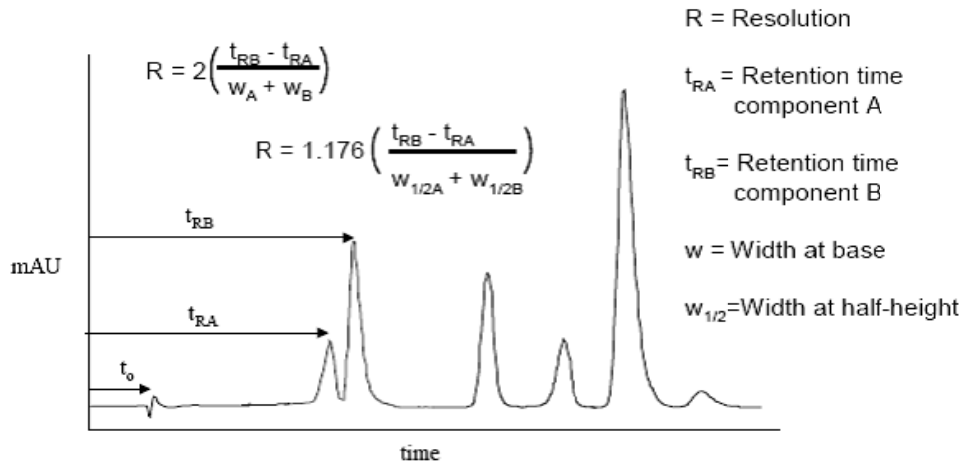
THEORY

Chromatography is an analytical method that finds wide application for the separation, identification and determination of chemical components in complex mixtures. This technique is based on the separation of components in a mixture (the solute) due to the difference in migration rates of the components through a stationary phase by a liquid mobile phase.

Following parameters are used in the chromatogram optimization –

1. Resolution
2. Capacity Factor
3. Efficiency Factor
4. Column Selectivity

Resolution



Resolution between Chromatographic Peaks is the primary concern in any analysis. Another goal is to accomplish this task in a minimum amount of time. The resolution between two peaks can be mathematically quantified with the equations appearing above. The first equation uses the width of the chromatographic peaks at their base. This width is found by drawing tangents through each side of the chromatographic peak. The second equation utilizes the width of the chromatographic peak at half-height. This value can be obtained from most chromatographic data systems as part of an integration or quantification report.

A resolution value of 1.5 between two chromatographic peaks of approximately equal peak height is considered baseline resolution.

Resolution Equation

This equation explains what factors affect the resolution

$$R = \frac{1}{4}\sqrt{N} \times \frac{\alpha - 1}{\alpha} \times \frac{k'}{1 + k'}$$

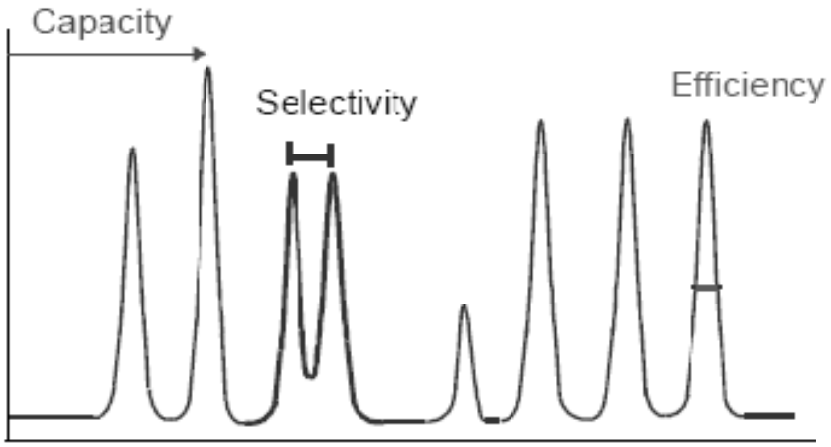
Efficiency *Selectivity* *Capacity*

N: Total number of theoretical plates available; used for column efficiency

k': Capacity factor (retention factor), the peak retention function

α: The relative separation of the peaks; the selectivity function

Resolution Factors



Efficiency, capacity, and selectivity are the three factors control that degree of resolution between two chromatographic peaks. The equation above expresses the role each of these components play in determining the resolution between the bands.

Capacity Factor

Also known as 'Separation factor'.The capacity factor is a measure of the retention of the sample molecule on the column. It represents the ratio of the elution time of the sample component to the void time of the column. $K' = (V_A-V_0)/V_0$This equation tells us how long it takes the centre of peak A to come off the column relative to void volume V_0.Molecules travel with the mobile phase unless they are interacting with the stationary phase. A molecule with absolutely no affinity for the stationary phase would elute in one column volume with the mobile phase solvent front and have a k' of 0

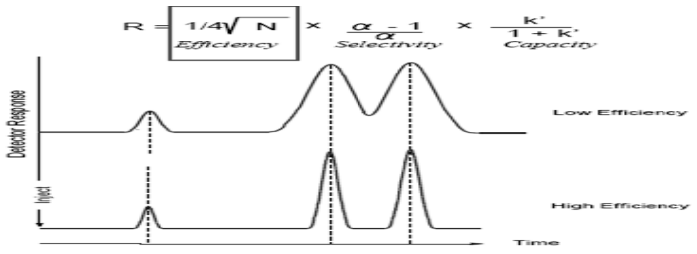
k' value ranges from 1-20.

k' = 0 implies that the compound is not retained and elutes with the solvent front

k' = 1 implies that compound is slightly retained by the column.

k' = 20 implies that compound is highly retained and spends much time interacting with stationary phase.

Efficiency Factor



It measures the degree of sharpness of a peak. Efficiency factor is determined by retention value of peak by peak width.

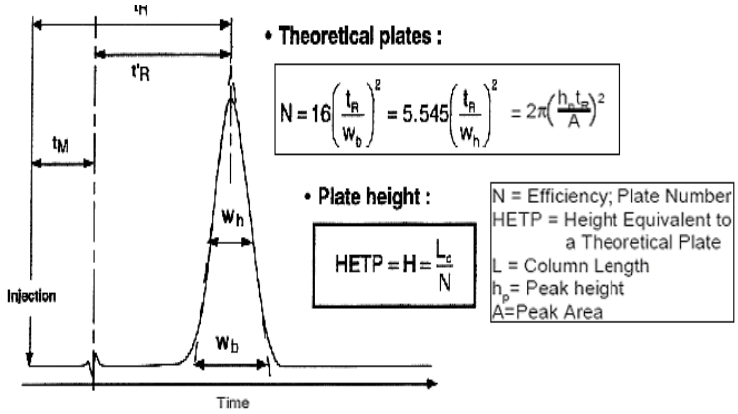
$$N = 16 (V_x / W_x)^2$$

Sharper the peak, better the separation and higher the efficiency of the column and system. In HPLC column, the larger the plate count, the sharper the peaks are and the smaller the peaks overlap between them.

Variables affecting efficiency are –

- Pump flow rate
- Extra column volumes in the instrument used
- Method of calculations

Calculating Efficiency



All the following methods use this formula that measures N, or number of theoretical plates:

$$N = a \frac{t_r^2}{W^2}$$

a = constant dependent on height where peak width measured

t_r = retention time

W = peak width

Column Selectivity

Column selectivity, α, is a measure of the relative separation of two peaks and is defined as the ratio of the net retention times of the two peaks.

$\alpha = (tm_2 - tm) / (tm1 - tm)$

tm = retention time of void volume

tm_1 = retention time of peak1

tm_2 = retention time of peak 2

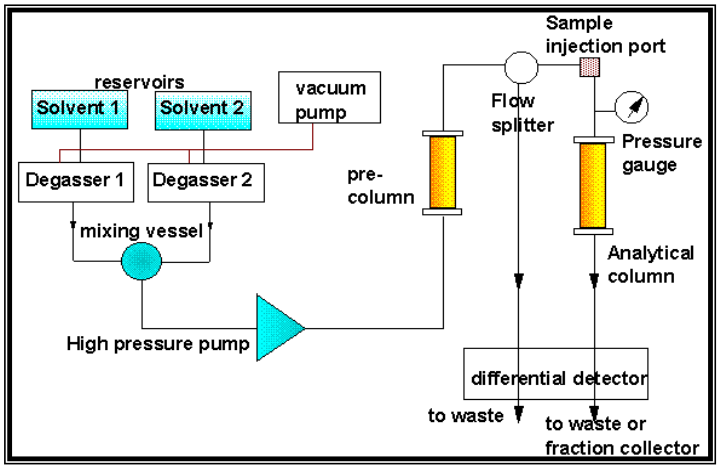
Separation factor α can be calculated by dividing the two k1's of two peaks. α value ranges from 1.0-2.0.

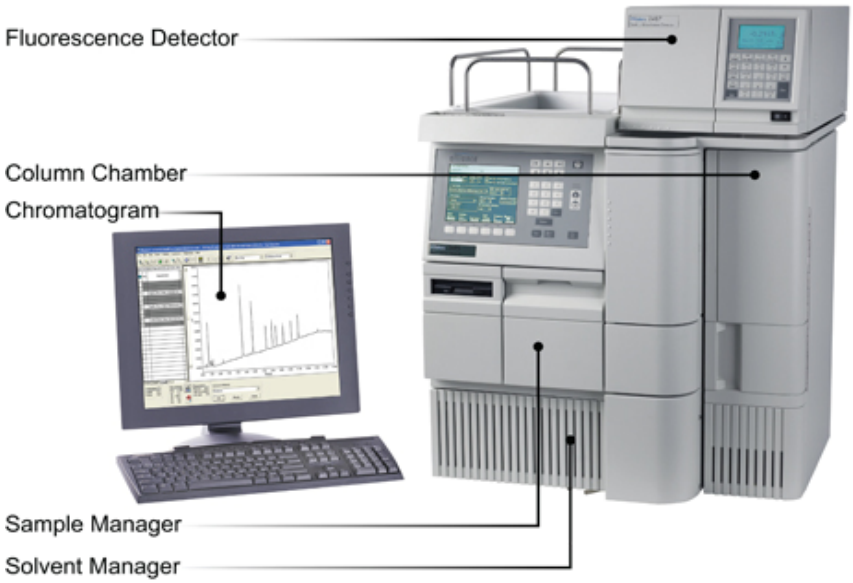
Components with α = 1.0 overlap completely and those with α > 2.0, can be separated by a separatory funnel. Larger α's are needed in HPLC only in preparative runs.

Ranges of chromatography parameters

S.No	PARAMETER	RANGE
1	Capacity factor (k')	
	Analytical	1-8
	Preparative	4-12
2	Efficiency factor (N)	Hundreds (poor resolution) to 10's of 1000's (good resolution)
3	Selectivity (or) Separation factor (α)	1-2. At α = 1, peaks overlap completely

INSTRUMENTATION





SYSTEM COMPONENTS

Solvent delivery system

The mobile phase is pumped under pressure from one or several reservoirs and flows through the column at a constant rate. With micro particulate packing, there is a high-pressure drop across a chromatography column. Eluting power of the mobile phase is determined by its overall polarity, the polarity of the stationary phase and the nature of the sample components. For normal phase separations eluting power increases with increasing polarity of the solvent but for reversed phase separations, eluting power decreases with increasing solvent polarity. Optimum separating conditions can be achieved by making use of mixture of two solvents.

The solvent delivery system has three basic functions:

- Provide accurate and constant flow through the flow channel.
- Provide accurate mobile phase compositions.
- Provide the force [pressure] necessary to push the mobile phase through the tightly packed column.

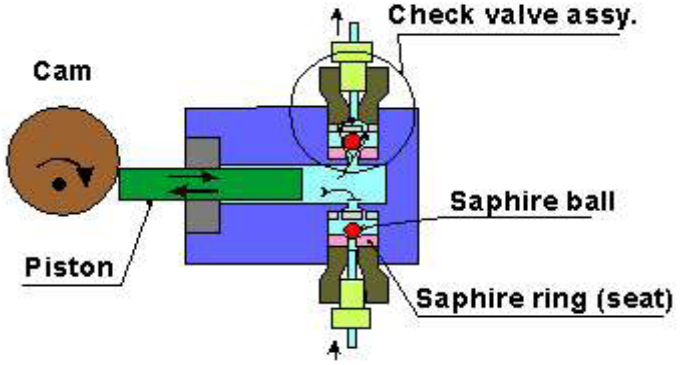
A solvent delivery system must provide accurate, reproducible flow and composition. System must also provide the force necessary to push the mobile phase through the tightly packed column. In addition, the solvent delivery system can't produce pressure pulsations. So damping unit is usually customary.

Damping units

The purpose of the damping unit is to reduce pressure pulsations caused by the action of the pump. A damping unit consists of a diaphragm separating the mobile phase from a compressible liquid.

Pump

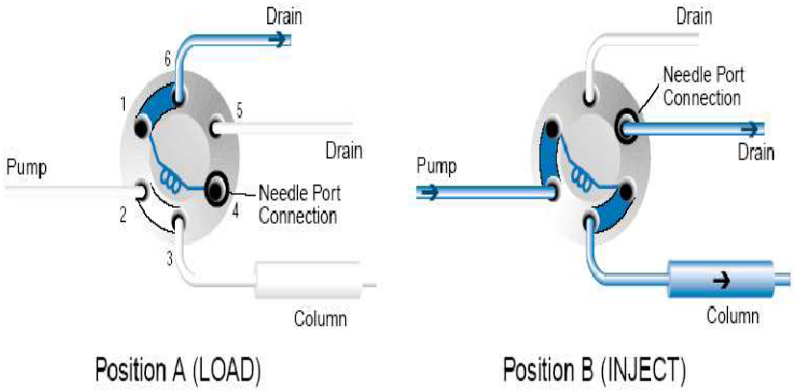
The most important component of HPLC in solvent delivery system is the pump, because its performance directly effects the retention time, reproducibility and detector sensitivity.



SAMPLE INTRODUCTION SYSTEM

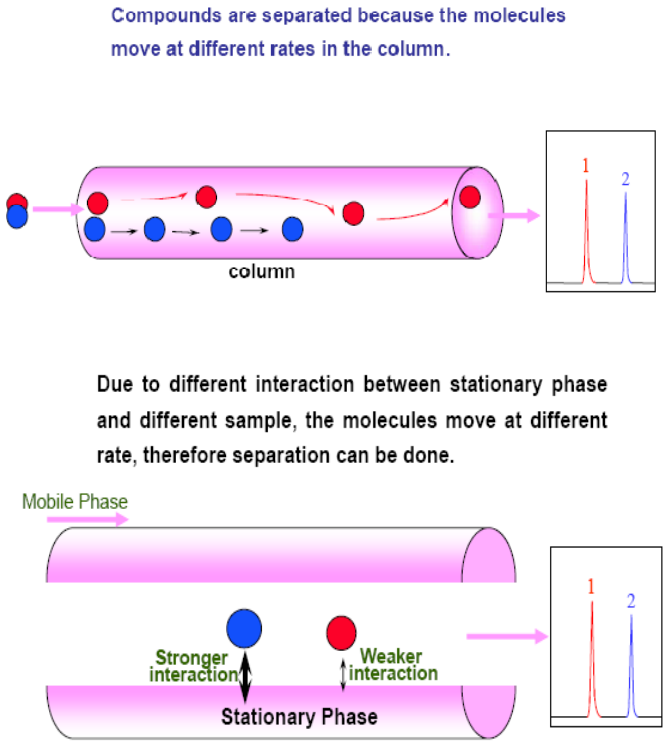
A sample introduction system is required to deliver the sample to the head of the HPLC column. The sample must be delivered without stopping or disturbing the mobile phase flow to the column. Sample injector must be very accurate and precise in its delivery. The sampler must also display low memory effects (carry-over).

Injectors are used for reproducible introduction of the sample volume into the mobile phase flow.



Column

Many separations depend not only on the column material and mobile phases but also on the column Temperature. In such cases, column temperature stability is the dominating factor for the elution order. The heart of the system is the column. In order to achieve high efficiency of separation, the column material (micro-particles, 5-10 µm size) packed in such a way that highest numbers of theoretical plates are possible.



Silica (SiO2 X H2O) is the most widely used substance for the manufacture of packing materials. It consists of a network of siloxane linkages (Si-O-Si) in a rigid three dimensional structure containing inter connecting pores. The silanol groups on the surface of silica give it a polar character, which is exploited in adsorption chromatography using non-polar organic eluents. Silica can be drastically altered by reaction with organo chloro silanes or organo alkoxy silanes giving Si-O-Si-R linkages with the surface. The attachment of hydrocarbon change to silica produces a non-polar surface suitable for reversed phase chromatography where mixtures of water and organic solvents are used as eluents. The most popular material is octadecyl-silica (ODS-Silica), which contains C_{18} chains, but materials with C_2, C_6, C_8 and C_{22} chains are also available.

During manufacture, such materials may be reacted with a small mono functional silane (e.g. trimethyl chloro silane) to reduce further the number of silanol groups remaining on the surface (end-capping).

Detectors

The detector for an HPLC is the component that emits a response due to the eluting sample compound and subsequently signals a peak on the chromatogram. It is positioned immediately posterior to the stationary phase in order to detect the compounds as they elute from the column. The bandwidth and height of the peaks may usually be adjusted using the coarse and fine tuning controls, and the detection and sensitivity parameters may also be controlled (in most cases). There are many types of detectors that can be used with HPLC. There is no one highly sensitive, universal detector system used for HPLC. The system used is thus based on the requirements which need to be met such as detection limits, expense etc

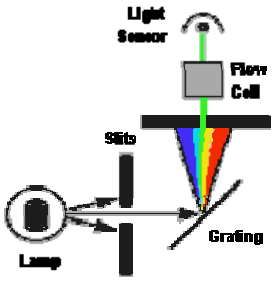
A summary of various detection methods are as follows

Spectroscopic detection methods

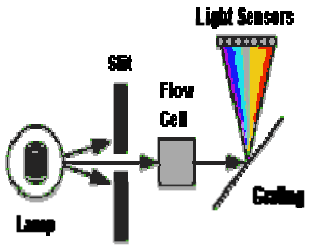
Molecular spectroscopic techniques

- UV Detectors
- Refractive Index Detectors
- Fluorometric Detection
- Atomic Spectroscopic Techniques

Variable Wavelength UV Detector



Photodiode Array Detector



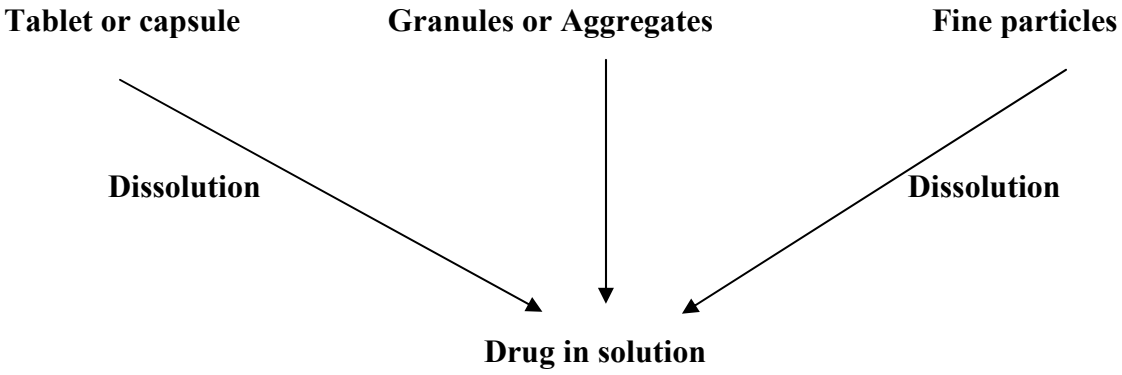
DISSOLUTION [6, 7, 8]

Dissolution is a process in which a solid substance solubilizes in a given solvent that is mass transfer from the solid phase to the liquid phase. Pharmaceutical solid dosage forms and solid-liquid dispersed forms, on administration, under dissolution in biological media, followed by absorption of drug entity into systematic circulation. In determining the dissolution rate of drug from solid dosage forms, under standardized conditions, one has to consider several physicochemical processes in addition to the process involved in the dissolution of pure chemical substances.

The following factors that influence the dissolution characteristic of the drug.

- Physical characteristics of dosage form
- Wet ability of the dosage unit
- Penetration ability of the dissolution medium
- Swelling process
- Disintegration and disaggregation of the dosage form

Wagner proposed the scheme regarding the process involved in the dissolution of solid dosage forms is given as follows:



The Wagner scheme was later modified to incorporate other that precedes the dissolution process of the solid dosage forms. Cartesian proposed a scheme incorporating the following sequence.

1. Initial Mechanical Lag
2. Wetting of the dosage form
3. Penetration of the dissolution medium into dosage form

4. Disintegration
5. Disaggregation ion of the dosage form
6. Dissolution
7. Occlusion of some particles of the drug.

It is apparent from the Wager's schematic representation that the rate of dissolution of the drug can become the rate-limiting step before the drug appears in blood. When the dosage form is placed into gastrointestinal tract in solid form, for the drug enters to the blood stream, there are two possible rate-limiting steps.

1. Freely purified water-soluble drugs will tend to dissolve rapidly, making the passive diffusion of the drug and or the active transport of the drug as the rate-limiting step for the drug to enter the blood stream.
2. Conversely, the rate of absorption of poorly soluble drug will be limited by the rate of dissolution of the drug or disintegration of dosage form.

Discriminative dissolution method

A method that is sensitive to change in formulation, raw material characteristics and critical manufacturing variable is said to be discriminative dissolution method.

In official or QC release method, the two products may shows same dissolution profile but when discriminative dissolution method is used, we can differentiate the formulation profile by release profile Once a discriminative dissolution method is developed, an in vitro-in vivo correlation can be established which could forecast the bioavailability of all the formulation developed during the product development. Factors like type of apparatus used, rpm, volume of dissolution media should be considered for the development of discriminative dissolution method. For study, we have selected rpm as one of the important factor for discriminative dissolution method.

The important parameters for the development of dissolution methods are

1. The pH dependent solubility
2. Stability

Applications

Discriminative dissolution method is an important tool in prototype formulation and development. Its applications are as follows:

It cans differentiate the change in formulation, which is due to variation in manufacturing process like granulation time, mixing time etc.

It predicts in-vivo behavior.

Once in vitro – in vivo correlation (IVIVC) is established, bioavailability study can be avoided, which can save the time and money in research work.

Theories of dissolution

Several theories to explain drug dissolution have been proposed. Some of the important ones are:

1. Diffusion layer model / Film theory
2. Danckwert's model / Penetration or Surface renewal theory, and
3. Interfacial barrier model / Double barrier or Limited solvation theory.

Classification of dissolution testing devices

1. USP Apparatus I (Basket Type)
2. USP Apparatus II (Paddle Type)
3. USP Apparatus III (Reciprocating Cylinder)
4. USP Apparatus IV (Flow-Through Cell)
5. USP Apparatus V (Paddle over disk)
6. USP Apparatus VI (Cylinder)
7. USP Apparatus VII (Reciprocating Holder)

Dissolution medium

The volume of the dissolution medium is generally 500, 900, or 1000mL. Sink conditions are desirable but not mandatory. An aqueous medium with pH range 1.2 to 6.8 (ionic strength of buffers the same as in USP) should be used. To simulate intestinal fluid (SIF), a dissolution medium of pH 6.8 should be employed. A higher pH should be justified on a case-by-case basis, and in general, should not exceed pH 8.0. To simulate gastric fluid (SGF), a dissolution medium of pH 1.2 should be employed without enzymes. The need for enzymes in SGF and SIF should be evaluated on case-by-case basis and should be justified. Recent experience with gelatin capsule products indicates the possible need for enzymes (pepsin with SGF and pancreatic with SIF) to dissolve pellicles, if formed, to permit the dissolution of the drug. Use of water as a dissolution medium also is discouraged because test conditions such as pH and surface tension can vary depending

on the source of water and may change during the dissolution test itself, due to the influence of the active and inactive ingredients. For water insoluble or sparingly water soluble drug products, use of a surfactant such as sodium lauryl sulfate is recommended (Shah, 1995). The need for and the amount of surfactant should be justified. Use of a hydro alcoholic medium is discouraged.

Agitation

In general, mild agitation conditions should be maintained during dissolution testing to allow maximum discriminating power and to detect product with poor in vivo performance. Using the basket method, the common agitation (or stirring speed) is 50-100 rpm; with the paddle method, it is 50-75 rpm.

FACTORS AFFECTING DISSOLUTION EXTENT

Equation [1] describes factors controlling extent of dissolution.

Maximum Dissolvable Dose = V × C_s / sink [1]

Where,

V = Dissolution medium volume

C_s = Saturated solubility of the compound in the medium

Sink = Sink condition factor

To increase the maximum dissolvable dose, one needs to increase the dissolution media volume, change the media to increase the saturation solubility of the compound, or reduce the dissolution sink requirements.

Media volume

There are several ways to increase the dissolution media volume. Using 4- liter vessel is relatively uncommon, but they are available from vendors. This offers a potential 4-fold enhancement in maximum dissolvable dose over the standard 1-liter vessels.

Saturation solubility

The standard way to affect the saturation solubility of the drug in the dissolution media is to change the media, typically by adjusting the pH, adding a surfactant, or in rare cases, using non-aqueous solvents.

pH

If the compound is ionizable, adjusting the pH of the dissolution media is a very effective way to increase solubility.

Surfactants

Two factors to consider when evaluating surfactants are cost and concentration needed. If the dissolution assay is to be run in a Quality Control setting, choosing an inexpensive surfactant will be important to keep overall assay costs down. Examples of inexpensive surfactants are sodium dodecyl sulfate or SDS (also referred to as sodium lauryl sulfate or SLS) for an anionic surfactant, cetyltrimethylammonium bromide or CTAB for a cationic surfactant, and the polysorbates or Tweens for a non-ionic surfactant. To get any substantial solubility enhancement, the surfactant concentration must be at least above the critical micelle concentration or CMC. The CMC will depend upon, among other things, the surfactant itself and the ionic strength of the media. The amount of surfactant needed depends on the CMC and the degree to which the compound partitions into the surfactant micelles.

If the compound is ionizable, surfactants concentration and pH may be varied simultaneously, and the combined effect can substantially change the solubilization ability of the dissolution media

Non-aqueous solvents

The use of non-aqueous solvent for dissolution media is unconventional. However, if aqueous-based methods for achieving solubility have been exhausted, use of hydro-alcoholic media may be the best alternative. For example, the USP24-NF19 monograph for cortisone acetate tablets lists 30% isopropanol, 70% 0.01 N HCl as the dissolution media, and water/alcohol mixtures have been used as media for drug release testing of topical formulations using the Franz-diffusion cell apparatus.

Sink conditions

Sink condition refer to the excess solubilizing capacity of the dissolution medium. Most sources recommend at least 3X (three times the volume needed to completely solubilize the dose) and some sources recommend 5X and even 10X.

METHOD DEVELOPMENT [9, 10, 11]

Every day many chromatographers face the need to develop a high –performance liquid chromatographic separation. Whereas individual approaches may exhibit considerable diversity, the method development often follows the series of steps summarized.

There exists today a good practical understanding of chromatographic separation and how it varies with the sample and with experimental conditions .Any systematic approach to HPLC method development should be based on the knowledge of the chromatographic process. In most cases a desired separation can be achieved easily with only a few experiments .In other cases, a considerable experimentation is needed. A good method development strategy should require as many experimental runs as are necessary to achieve the desired final result.

STEPS IN HPLC METHOD DEVELOPMENT

1. Information on sample, and separation goals
2. Need for special HPLC procedure
3. Choose detector and detector settings
4. Choose LC method; preliminary run; estimate the best separation conditions.
5. Optimize separation conditions.
6. Check for problems
7. Recover purified material
    a. Quantitative calibration
    b. Qualitative method
8. Validate method to release to routine laboratory.

WHATS IS KNOWN BEFORE STARTING

NATURE OF THE SAMPLE

- Number of compounds present
- Chemical structure of compound
- Molecular weight of compound
- pKa values of compound
- UV spectra of compound
- Concentration range
- And sample solubility.

Separation goals

Is the primary goal quantitative analysis, the detection of an substance, the characterization of unknown sample components (or) isolation of purified material.

Is it necessary to resolve all sample components

SAMPLE PRETREATMENT AND DETECTION

Sample comes in various forms

- Solutions ready for injection
- Solids that must first be dissolved or extracted
- Samples that require sample pretreatment to remove interferences and/or protect the column or equipment from damage.
- Solutions that require dilution, buffering, addition of internal standard or other volumetric manipulation.

Most samples for HPLC analysis require weighing and/ or volumetric dilution before injection.

Some samples require a partial separation (pretreatment) prior to HPLC because of need to remove interferences, concentrate sample analyte, or eliminate column killers. This means that it is important to know the nature of the sample matrix and the probable concentrations of various analyte, in many cases the development of an adequate sample pretreatment procedure can be more challenging than achieving a good HPLC separation. Finally, method precision and accuracy are frequently determined by the sample pretreatment procedure. A sample pretreatment procedure should provide quantitative

recovery of analyte, involves a minimum number of steps, and be easily automated. Quantitative (99+ %) recovery of each analyte enhances sensitivity and assay precision.

PRELIMINARY PROCESSING OF SOLID SAMPLES

Reducing sample particle size

It is desirable that solid samples be reduced in particle size since finely divided samples are

1. More homogenous , allowing more representative sampling with greater precision and accuracy and
2. Dissolve faster and are easier to extract because of their greater surface area.

Methods for reducing sample particle size are blending, chopping, crushing, grinding, homogenizing, macerating, milling, mincing, pressing, pulverizing, sieving.

EXTRACTION

Extraction methods for solid samples

- Solid –liquid extraction
- Soxhlet extraction
- Forced-flow leaching
- Homogenization
- Sonication
- Dissolution
- Accelerated solvent extraction
- Automated soxhlet extraction
- Supercritical fluid extraction
- Microwave-assisted extraction
- Thermal extraction

DRYING THE SAMPLE

Solid samples are often received for analysis in a damp or wet mass. Removal of water or drying the sample to constant weight is usually necessary for reliable assay.

Hydrophobic organic samples require heating, since water absorption is minimal, for **hygroscopic** or reactive samples, drying in vacuum desiccators is recommended. Freeze drying (lyophilization) often used to preserve the integrity of heat sensitive samples (especially biological)

FILTRATION

Particulates should be removed prior to injection because of their adverse effect on column life. The most common methods for removing of particulates from the sample are filtration, centrifugation, and sedimentation. A variety of membrane materials and different nominal porosities and dimensions are available for filtration. For most samples encountered in HPLC, filters in the range 0.25-2.0µm nominal porosities are recommended .Membranes with 0.25µm pores remove the smallest of particulates.

DETECTION

Before the first sample is injected during HPLC method development we must be reasonably sure that the detector selected will sence all sample components of interest. Variable wavelength ultraviolet detectors normally are the first choice, because of their convenience and applicability for most samples. For this reason, information on the UV spectra can be an important aid for method development.UV spectra can be found in the literature, estimated from the chemical structures of sample components of interest, measured directly or obtained during HPLC separation by means of a photo diode array (PDA) detector. When the UV response of the sample is inadequate, other detectors are available (fluorescence, electrochemical, etc,)

GETTING STARTED ON METHOD DEVELOPMENT

The only remaining decision before the first sample injection is the percent organic in the mobile phase (%B). One approach is to use an isocratic mobile phase of some average solvent strength (eg.,50%B).A better alternative is to use a very strong mobile phase first (eg., 80 to 100% B) ,then reduce the % B as necessary. An alternative to initial isocratic separation is the use of gradient elution.

IMPROVING THE SEPARATION

The separation achieved in the first one or two runs usually will be less than adequate. After a few additional tries, it may be tempting to accept a marginal separation, especially if no further improvement is observed .However experienced workers realize that a good separation requires more than minimal resolution of the individual sample bands, particularly for a routine procedure used to analyse a number of samples.

SEPARATION GOALS IN HPLC METHOD DEVELOPMENT

Resolution – precise and rugged method quantitative analysis requires that Rs be greater than 1.5

Separation time- < 5 -10 min is desirable for routine procedures

Quantitation - ≤ 2% (1 SD) for assay, ≤ 5%for less-demanding analyses, ≤ 15% for trace analysis.

Pressure- < 150 bar is desirable, < 200 is usually essential (new column assumed)

Peak height-narrow peaks are desirable for larger signal/noise ratios.

Solvent consumption- minimum mobile phase use per run is desirable

REPEATABLE SEPARATION

It is important to confirm that each chromatogram can be repeated, as the experimental runs described being carried out. When changing conditions (mobile phase, column, temperature) between method development experiments, enough time must elapse for the column to come in to equilibrium with the new mobile phase and temperature .Usually column equilibrium is achieved after passage of 10 to 20 column volumes of the mobile phase through the column. However, this should be confirmed by carrying out a repeat experiment under the same conditions. When constant retention times are observed in two such back-to–back experiments (±0.5% or better)Column equilibration can be extremely slow for certain reversed-phase HPLC conditions ; addition of basic modifiers or ion pair reagents to the mobile phase, the use of Tetrahydrofuran as solvent or the use of mobile phase without organic solvent.

COMPLETING THE HPLC METHOD

The final procedure should meet all the goals that were defined at the beginning of method development. The method should also be robust in routine operation and usable by all laboratories and personnel for which it is intended.

Completing the method

1. Preliminary data to show required method performance.
2. Written assay procedures developed for use by other operators.

3. Systematic validation of method performance for more than one system or operator , using samples that cover the expected range in composition and analyte concentration; data obtained for day –to – day and interlaboratory operation.
4. Data obtained on expected life of column and column-to-column reproducibility.
5. Deviate results studied for possible correction of hidden problems.
6. All variables (temperature, mobile phase, composition, etc) studied for effect on separation; limits defined for these variables; remedies suggested for possible problems (poor resolution of key band pair, increased retention for last band with longer run times).

CHECKING FOR PROBLEMS

Low plate number – poor choice of column, secondary retentions, poor peak shape.

Column variability- poor choice of column, secondary retentions effects.

Short column life- poor choice of column, need for sample pretreatment ,3> pH > 7.

Retention drift –insufficient column equilibration, need for sample pretreatment, loss of bonded phase.

Poor quantitative precision- need for better calibration, identification of sources of error.

New interference peaks discovered- initial separation inadequate or initial samples not representative.

METHOD RUGGEDNESS

A rugged method is one that tolerates minor variations in experimental conditions, can be run easily by an average chromatographer, and does not require an identical HPLC system for its use. Rugged methods are essentially trouble free and transferable. Ruggedness can also be designed into a method by studying the effects of different variables on the separation.

METHOD VALIDATION[13, 14]

Then word validation simply means, "Assessment of validity" or action of proving effectiveness. "The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose."

-ICH Guideline Federal

Method Validation = Error assessment

Significance of method validation

The quality of analytical data is a key factor in the success of a drug development programme. The process of method development and validation has a direct impact on the quality of these data.

- To trust the method.
- Regulatory requirement.

Method validation is required for the following reasons

- Assuring quality
- Achieving the acceptance of the product by International Agencies
- Mandatory requirement purpose for accreditation as per ISO 19025 guidelines.
- Mandatory requirement for registration of any pharmaceutical product or pesticide formulation
- Validated methods are only applicable for proficiency testing.

Analytical methods should be validated unless the method employed is included in the relevant pharmacopoeia or other recognized standard reference.

VALIDATION PARAMETERS

SPECIFICITY

Specificity is the ability to asses unequivocally the analyte in the presence of components, which may be expected to be present.

LINEARITY

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample. In order to determine the quantity of any analyte present in unknown sample, some kind of relationship (mathematical/empirical) between concentration and response was essential. Response should be direct proportion to the concentration.

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

LOD

Lowest amount of analyte in a sample that can be detected but not necessarily quantities, under the stated experimental conditions (LOD).

LOQ

Lowest amount of analyte in a sample, which can be quantitatively, determined with suitable precision and accuracy (LOQ). SD of response (σ) & Slope(S): Linearity curve was prepared with a series of working standard solutions at different concentrations (3 concentrations below 50% of specification level and 3 more concentration above 50% specification level were performed)

RSD CRITERIA

Series of working standard solutions of different concentrations below to specification level were prepared (generally about 10%, 20%, 30%, 40% and 50% of the specification concentration) and injected six replicate injections into HPLC. Precision should be established (if predicted from other than RSD criteria) at LOQ and LOD level as per ICH, USP& EP guidelines. The solution was prepared at predicted concentration (for LOQ/LOD) and injected six replicates as per methodology.

ACCURACY

The accuracy of an analytical procedure express closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found.

Accuracy was usually determined in one of four ways:

- The procedure was applied to the known concentration of reference sample and the measured value to the true value was compared (defined by the organization, from which the sample received)
- The test results obtained were compared by the analytical procedure which was proved to be accurate with the results obtained from an existing alternate method that was known to be accurate
- Spiking concept, by spiking either analyte/impurities into sample matrix was the other

Recovery concept-standard addition approach. This approach was applicable, if it was not possible to prepare a blank sample matrix without the presence of the analyte

PRECISION

Precision is the measurement of how close the data values to each other for a number of measurements under the same analytical conditions. Precision may be considered at three levels according to ICH

- Repeatability
- Intermediate precision
- Reproducibility

REPEATABILITY

Precision under same operative conditions (with- in a laboratory over a short period of time using the same analyst with the same equipment)

Measurement/ Injection repeatability (system precision).

Method repeatability (Method precision)

INTERMEDIATE PRECISION (RUGGEDNESS)

Precision under different laboratory conditions (within-laboratory variation, as on different days, or with different analysts, or equipment's within the same laboratory

REPRODUCIBILITY

Precision between laboratories/intermediate precision can be considered during the standardization of a procedure before it i.e. submitted to the pharmacopoeia.

RANGE

The range of an analytical procedure is the interval between the upper and lower concentrations (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The range of an analytical procedure was the concentration interval over which acceptable accuracy, precision and linearity were obtained. In practice, the range was determined using data from the linearity and accuracy studies. Assuming that acceptable linearity and accuracy (recovery) results were obtained as described earlier. The only remaining factor o be evaluated was precision. This precision data should be available from the triplicate analysis of spiked sample sin accuracy study. Hence to confirm the "range" of any analytical procedure, linearity studies alone not sufficient and accuracy at each concentration (minimum three concentration levels covering lower and upper levels) should be proved.

ROBUSTNESS

Measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides indication of its reliability during its normal usage. Varying method parameters within a realistic range and the quantitative influence of the variables was determined, and, if the influence of the parameter was within a previously specified tolerance, then, the parameter was said to be within the method's robustness range.

Typical variations included under Validation programme were

- Flow rate (0.1%)
- pH of the mobile phase(0.1 unit)
- Temperature (2ºC)
- % of Organic solvent (2%)
- Wavelength (2 nm)

RUGGEDNESS

Method Ruggedness is defined as the reproducibility of results when the method is performed under actual use conditions. This includes different analysts, laboratories, columns, instruments, source of reagents, chemicals, solvents etc. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method.

STABILITY

To generate reproducible and reliable results, the samples, standards and reagents used for the HPLC method must be stable for a reasonable time (e.g. one day, one week and one month depending upon need). For example, the analysis of even a single sample may require ten or more chromatographic runs to determine the system suitability, including standard concentrations to create a working analytical curve and duplicate or triplicate injections of the sample to be assayed.

VALIDATION PARAMETERS FOR ASSAY AND DISSOLUTION

S. No	VALIDATION PARAMETER	ASSAY	DISSOLUTION
1	System Suitability and System Precision	✓	✓
2	Specificity	✓	✓
3	Precision	✓	✓
4	Accuracy	✓	✓
5	Linearity of Method	✓	✓
6	Ruggedness	✓	✓
7	Robustness	✓	✓
8	Filter Validation	✓	✓
9	Sink condition	X	✓

SINK CONDITION

Sink condition refers to the excess solubilizing capacity of the dissolution medium.

2. LITERATURE REVIEW

1) **Hairong Wang[15] et al** has been developed a sensitive and specific liquid chromatography–tandem mass spectrometric (LC–MS–MS) method to determine *m*-nisoldipine in rat plasma. Sample was pretreated by a single-step protein precipitation with acetonitrile, in contrast to the liquid–liquid procedure frequently used for the extraction of 1, 4-dihydropyridines from biologic samples. Separation of analyte and internal standard (I.S.) was performed on Symmetry RP-C18 analytic column (50mm×4.6 mm, 3.5_m) with a mobile phase consisting of acetonitrile–water (80:20, v/v) at a flow rate of 0.5 ml/min.The API 4000 triple quadrupole mass spectrometer was operated in multiple reaction monitoring (MRM) scan mode using TurboIonSpray ionization(ESI) source. This method was sensitive with a lower limit of quantification (LLOQ) of 0.2 ng/mL, with good linearity ($r \geq 0.9982$) over the linear range of 0.2–20 ng/mL

2) **R Heinig have developed[16] et al** a method for Determination of the enantiomers of nisoldipine in human plasma using high-performance liquid chromatography on a chiral stationary phase and gas chromatography with mass selective detection .this method has described that combines chiral HPLC and off-line GC with mass-selective detection for the quantitation of the enantiomers of nisoldipine [(+)-I] in human plasma. An isotope-labeled internal standard [nine-fold deuterated (+)-I] is used throughout the assay. The limit of quantification is 0.1 pg/l for each enantiomer. Enantioselective analysis was performed in subjects receiving the racemic drug in tablet form. In healthy volunteers the maximum Concentration and the area under the curve of the pharmacologically more active (+)-enantiomer were greater by 9-fold and 13-fold, respectively, compared to those of the (-)-enantiomer. After intravenous administration of (+)-I there were no relevant differences between the plasma concentrations of the enantiomers.

3) **H Sugawara[17] et al** has studied Antioxidant Properties of Dihydropyridine in Isolated Sprague-Dawley rat hearts were perfused under constant flow conditions. Hearts were treated with H_2O_2 (500-600 PM), as buffer for 12 min including nisoldipine, nifedipine, or the optical isomers (+) - or (-)-nisoldipine. H_2O_2, was removed and perfusion continued with treatment buffers (10 min) followed by control buffer (20 min). Contractile function decreased following

perfusion with H_2O_2. Contractile function was protected in a concentration-dependent Manner (nisoldipine = 19, 26, 50, 63, and 78%; nifedipine = 23, 37, 55, 61, and 63% of pre-peroxide function, 0, 0.1, 0.5, 1.0, and 5 nM, respectively). There were no significant differences between equal concentrations of nisoldipine and nifedipine. Contractile function was equally protected by both (+) - and (-)-nisoldipine compared with vehicle-treated hearts (56, 67, and 16% of pre-peroxide function, respectively). Biochemical analyses indicated that H_2O_2, damaged plasma membranes (increased lactate dehydrogenase leak) and caused lipid per oxidation (elevated tissue thiobarbituric acid reactive substances). Biochemical changes were equally reduced by nisoldipine and nifedipine treatments and by (+) - and (-)-nisoldipine. The treatment groups have widely differing ICI**,** v, a lues as calcium channel antagonists, yet they had equal effects in reducing oxidative injury, suggesting that this beneficial effect is not mediated by calcium antagonism.

4) **M Gilar [18] et al** has studied Enantiomer separation of dihydropyridine calcium antagonists with Cyclodextrins as chiral selectors: structural correlation in high-performance liquid chromatography and capillary electro migration separations (HPCE and MEKC). Chromatographic data of five dihydropyridine calcium antagonists obtained on three/3-CD chiral stationary phases in reversed-phase mode. This data were compared with those of capillary Electrophoresis using /3-CDs in the presence and absence of sodium dodecyl sulfate (SDS). Competition of separated compounds with SDS molecules for penetration into the CD cavity can limit their necessary interaction with the chiral selector and consequently even preclude enantiomer separation.

5) J Mielcarek.[19] et al Inclusion complexes **of nifedipine** and other 1.4-dihydropyridine derivatives with Cyclodextrins. IV. The UV study on photochemical stability of the inclusion complexes of nisoldipine, nimodipine, nitrendipine and nicardipine with beta-Cyclodextrins in the solution. J Chromatogr A. 666 (1-2); 241-8(1994)

6) **D Zimmer[20] et al** have developed a sensitive, selective and validated method for the enantioselective determination of (+) - and (-)-nisoldipine in rat, mouse and dog plasma following administration of nisoldipine racemate by chiral microbore high-performance liquid chromatography Combined with gas chromatography-mass spectrometry. The enantiomers of nisoldipine were quantitatively separated by high-performance liquid chromatography on a 250 x 2 mm I.D. column containing tris (4_methylbenzoate) - modified cellulose on silica. The fractions containing either the (+) or (-)-enantiomer of the analyte and [$^{13}C_4$] ISTD were Analyzed by gas chromatography with mass-selective detection in the single-ion monitoring mode. The limits of determination and detection were 0.5 and 0.2 ng/ml, respectively, the total precision was 7% (R.S.D. at 5 and 50 ng/ml, n = 35) and the accuracy was 10% (0.5-100 ng/ml, n = 23). The sum of the concentrations of the enantiomers determined with this assay corresponds to the concentration of the racemate determined independently by capillary gas chromatography with Electron-capture detection (accuracy better than 15%, l-80 ng/ml).

7) **A Alvarez-Lueje[21]** et al The study by dc and d.p.p. reveals the appearance of four signals depending on pH. In contrast, the anodic response corresponds to the oxidation of the 1, 4-dihydropyridine ring to generate the corresponding pyridine derivative. Both, cathodic (d.p.p.) and anodic signals (d.p.v.) were employed to develop analytical methodology for the determination of the drug. The repeatability of the measurements for both methods was adequate with R.S.D. of 1.4% (*n*_10) and 2.1% (*n*_10) for d.p.p. and d.p.v., respectively. Also recovery studies, 103.8% (R.S.D. 2.65%) by d.p.p. and 98.7% (R.S.D. 2.1%) by d.p.v. show that the accuracy and precision of the developed methods were adequate. The analytical methods were successfully applied to the determination of nisoldipine in both tablets and capsules. In addition, a preliminary study of the photo stability of nisoldipine (using both UV and artificial day light) was completed. The identity of the main electro active photo degradation products by GC with spectrometry detection is provided.

8) **V Marinkovic[22] et al** have studied photochemical degradation of Nisoldipine ((9)3-isobutyl-5-methyl-1, 4-dihydro-2, 6-dimethyl-4-(2-nitrophenyl)-pyridine-3, 5-dicarboxylate), whereby its 4-(2-nitrosophenyl) pyridine analogue is obtained as the photolytic product, under daylight exposure by means of UV derivative spectrophotometry. The optimal instrumental parameters

(120 nm:min scan speed; 2 nm slit width; Dl_10 nm and 5 s response time) for analogue derivative spectra were established for amplitudes 1*D*285 and 2*D*291(measured to the baseline) of the nitroso analogue assay, as well as for 1*D*386 of the parent compound–nisoldipine assay. Using the first-order derivative spectrum, the minimum detectable amount of nitroso analogue in the presence of nisoldipine was equivalent to an impurity level of 5% and by the second-order derivative spectrum; the determination limit was equivalent to an impurity level of 2%. The degradation of nisoldipine followed within 30 days and the calculated maximal degradation rate was1.6% per day for nisoldipine raw material, but significantly lower values of 0.19 and 0.15% per day were obtained for Nisoldin®tablets (10 and 5 mg, respectively)

9) **Valentina D Marinkovic[23] et al** has studied photochemical degradation of solid-state nisoldipine, 1, 4-dihydropyridine calcium antagonist, under daylight and UV light conditions. Degradation products were identified by using the retention times of corresponding standards and quantified by high-performance liquid chromatographic method. HPLC experiments were carried out on a Hewlett Packard LC 1100 instrument, equipped with binary solvent pump G 131 2A and variable detector G 1314A. An octadecyl silane column (Lichrosorb RP-18, 5 mm, 250_ 4 mm, Merck, Darmstadt, Germany) was used and methanol: water (60:40 v/v), pH 3.0 adjusted with phosphoric acid was used as mobile phase. Flow rate 1 ml/min and loop 20 ml were used. The samples were monitored at 238 nm. The daylight illumination induced appearance of nitrosophenylpyridine, while formation of second degradation product, nitrophenylpyridine, was observed only upon UV light illumination. The photo degradation kinetics of solid-state nisoldipine under daylight and UV light illumination belongs to class of zero-order reaction

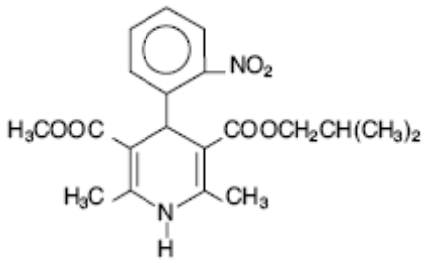
10) **Wonku kang[24]** et al has developed a simple and rapid quantification method for determining nisoldipine in plasma by Liquid Chromatography–Tandem Mass Spectrometry. After a simple protein precipitation with a mixture of 10% of zinc sulfate and methanol, the analytes were chromatographed on a reversed-phaseC18 column, and detected by MS/MS. The assay precision was less than 10.7%, and the accuracy ranged from 86 to112%. The limit of quantification was 0.1 ng/ml. This method was used to measure the plasma concentration of nisoldipine from healthy subjects after a single 5-mg oral dose of nisoldipine.

3. DRUG PROFILE[19,27]

DRUG NAME : NISOLDIPINE

Chemical Name : 3, 5-pyridinedicarboxylic acid, 1, 4-dihydro-2, 6-Dimethyl-4-(2-nitrophenyl)-, methyl 2-methyl- propyl ester

Chemical Structure:



Molecular formula	**:**	$C_{20}H_{24}N_2O_6$
Molecular weight	**:**	388.4
Description	**:**	yellow crystalline substance
Nature	**:**	1, 4-dihydropyridine derivatives
Melting point	**:**	143.22-152.52°C
Solubility	**:**	freely soluble in ethanol, chloroform Insoluble in water
Category	**:**	Antihypertensive Agents Vasodilator Agents Calcium Channel Blockers

Substructures

- Dihydropyridines
- Carboxylic Acids and Derivatives
- Nitrobenzene's
- Acetates
- Oxoazaniums
- Ethers
- Benzene and Derivatives
- Nitro compounds
- Enamines
- Heterocyclic compounds
- Aromatic compounds
- Anilines

Protein Binding **:** 99%

$T_{1/2}$ **:** 7-12 hours

Dose **:** 34 mg of Nisoldipine for once-a-day oral Administration

CLINICAL PHARMACOLOGY:

Mechanism of Action

By deforming the channel, inhibiting ion-control gating mechanisms, and/or interfering with the release of calcium from the sarcoplasmic reticulum, Nisoldipine inhibits the influx of extracellular calcium across the myocardial and vascular smooth muscle cell membranes The decrease in intracellular calcium inhibits the contractile processes of the myocardial smooth muscle cells, causing dilation of the coronary and systemic arteries, increased oxygen delivery to the myocardial tissue, decreased total peripheral resistance, decreased systemic blood pressure, and decreased after load.

Absorption and Distribution

Relatively well absorbed into the systemic circulation with 87% of the radio labeled drug recovered in urine and feces. The absolute bioavailability of Nisoldipine is about 5%.

Metabolism

Pre-systemic metabolism in the gut wall, and this metabolism decreases from the proximal to the distal parts of the intestine. Nisoldipine is highly metabolized; 5 major urinary metabolites have been identified. The major biotransformation pathway appears to be the hydroxylation of the isobutyl ester. A hydroxylated derivative of the side chain, present in plasma at concentrations approximately equal to the parent compound, appears to be the only active metabolite and has about 10% of the activity of the parent compound. Cytochrome P450 enzymes are believed to play a major role in the metabolism of Nisoldipine. The particular isoenzyme system responsible for its metabolism has not been identified, but other Dihydropyridines are metabolized by Cytochrome P450 IIIA4

.**Elimination**

Although 60-80% of an oral dose undergoes urinary excretion, only traces of unchanged Nisoldipine are found in urine. 87% of the radio labeled drug is recovered in urine and feces. Nisoldipine is eliminated 60% to 80% in urine (traces unchanged), 5 urinary metabolites and only 1 active

Pharmacodinamic

Nisoldipine, a Dihydropyridines calcium-channel blocker, is used alone or with an angiotensin-converting enzyme inhibitor, to treat hypertension, chronic stable angina pectoris, and Prinzmetal's variant angina. Nisoldipine is similar to other peripheral vasodilators. Nisoldipine inhibits the influx of extra cellular calcium across the myocardial and vascular smooth muscle cell membranes possibly by deforming the channel, inhibiting ion-control gating mechanisms, and/or interfering with the release of calcium from the sarcoplasmic reticulum. The decrease in intracellular calcium inhibits the contractile processes of the myocardial smooth muscle cells, causing dilation of the

coronary and systemic arteries, increased oxygen delivery to the myocardial tissue, decreased total peripheral resistance, decreased systemic blood pressure, and decreased after load.

Elderly

Higher Nisoldipine plasma concentrations (C_{max} and AUC) have been found in elderly.

Indications and Usage

Treatment of hypertension, alone or in combination with other antihypertensive agents.

Contraindications

Sensitivity to Dihydropyridines calcium channel blockers.

Dosage and Administration

Adults

PO Initiate therapy with 17 mg once daily, then increase by 8.5 mg/wk, or with longer intervals, to attain adequate BP control (max, 34 mg/day).

Patients older than 65 yr of age, or patients with impaired liver function

Initiate therapy with 8.5 mg once daily.

General Advice

- Have patient swallow tablets whole. Do not allow patient to crush, chew, or divide.
- Administer once daily 1 h before or 2 h after a meal. Do not administer with a high-fat meal. Avoid grapefruit products.

Storage/Stability

Store at 68° to 77°F. Protect from light and moisture.

4. OBJECTIVE AND PLAN OF WORK

4.1 OBJECTIVE

Among the various drugs currently available for the treatment of systematic hypertension, the calcium channel antagonists (CCAs) continue to receive much attention as a result of their benefits in the prevention of cardiovascular events and other complications. Nisoldipine as a new dihydropyridinecalcium ion antagonist.

Nisoldipine is chemically described as 3-isobutyl-5-methyl-1, 4-dihydro-2, 6-dimethyl-4-(2-nitro phenyl)-pyridine-3, 5-dicarboxylate, is a calcium-channel-blocking 1, 4-dihydropyridine derivative, with no identical ester functions, which has been developed as an antihypertensive and antianginaldrug.

A Stability indicating methods is a quantitative analytical procedure used to detect a decrease in the amount of the active pharmaceutical ingredient (API) present due to degradation. According to FDA guidelines, a Stability indicating methods is defined as a validated analytical procedure that accurately and precisely measures active ingredients (drug substance or drug product) free from potential interferences like degradation products, process impurities, excipients, or other potential impurities, and the FDA recommends that all assay procedures for stability studies be stability indicating .During stability studies, liquid chromatography (LC) is used routinely to separate and quantitate the analyte of interest.

Stability indicating methods are quantitative test methods that can detect changes with time of drug substances and drug products. Information of type and amount of degradation products over time is important for safety of drugs. Therefore, FDA and other agencies but also good business practice requires such methods to be well designed and validated.

Dissolution test has emerged in the pharmaceutical field as a very important tool based on the fact that for a drug to be absorbed and available to the systemic circulation, it must previously be solubilized. Therefore the dissolution studies are used not only to assess batch-to- batch consistency of drug release from solid dosage forms, but they are also essential in several stages of formulation development .The initial assay parameters may determined with drug substance dissolved in a dissolution medium. At the present time there is no dissolution test has been described in literature for this drug.

Method validation is an essential step in drug analysis. The process confirms that the analytical procedure employed for the analysis is suitable for its intended use and shows reliability of the results produced by any method.

The principal aspects of drug products that play an important role in shelf life determination of tablet formulation are assay and dissolution of active drug and degradants generated during the stability study. The assay of drug product in stability test sample needs to be determined using stability indicating method, as recommended by the International Conference on Harmonization (ICH) guidelines (ICH, 2000) and USP 29(United State Pharmacopoeia, 2005).

A thorough literature survey has revealed that only few analytical Methods that have been developed for its determination of Nisoldipine in human plasma has been Mainly determined using liquid or gas chromatography with mass spectrometry, following a Liquid–liquid extraction. And it has been analyzed for crystal structure Elucidation, and determination in formulations by voltametry, polarography.But there is no method has been developed for quantification for its formulation by High performance liquid chromatography. At the present time there is no dissolution test has been described in literature for this drug.

The main purpose of this investigation is to develop and validate simple, precise, sensitive and accurate stability indicating reversed phase high-performance liquid chromatographic method for assay and validate a sensitive RP-HPLC method to be applied to the in vitro dissolution studies.

Therefore, the present study has been undertaken in order to develop a new, simple, reproducible validatable, transferable, robust, reliable, accurate and precise individual methodology for the Assay and Dissolution of Nisoldipine in pharmaceutical dosage forms by using HPLC(Reverse phase).

4.2 PLAN OF WORK

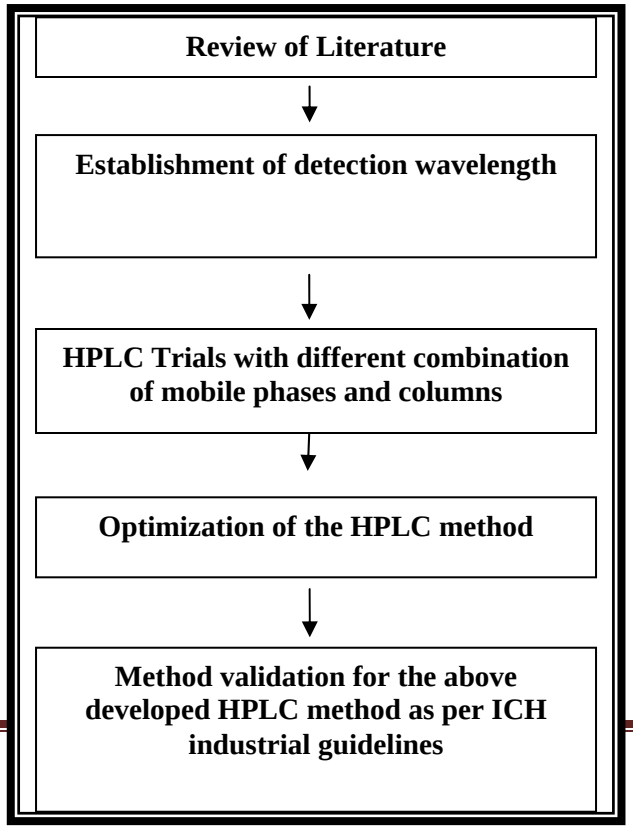
4.2.1 ASSAY METHOD DEVELOPMEMT

An attempt was made in a stepwise manner to develop a simple, rapid, selective and sophisticated Assay method, by High performance liquid chromatography (Reverse phase) for the Nisoldipine.

The following stepwise protocol was followed.

- As a start up, literature survey was done and from the literature survey chemical profile like solubility, chemical structure, pKa value and analytical profile were obtained.
- From the data obtained, UV spectroscopic study was tried in the first place.
- Later several trials were done in RP-HPLC using a different combination of mobile phases and finally optimized.
- After optimization of the HPLC method, validation of the analytical method for the developed RP-HPLC method was done in accordance with the ICH guidelines.

- The plan of work is presented in the following scheme



4.2.2 DISSOLUTION METHOD DEVELOPMENT

An attempt was made in a stepwise manner to develop a simple, rapid, selective and sophisticated Analytical method &Dissolution method, by High performance liquid chromatography (Reverse phase) for the Nisoldipine

The following stepwise protocol was followed.

- Various trail was made to find out the solubility of drug in Water,0.1N HCL, PH 6.8 Phosphate Buffer , and finally selected the suitable medium

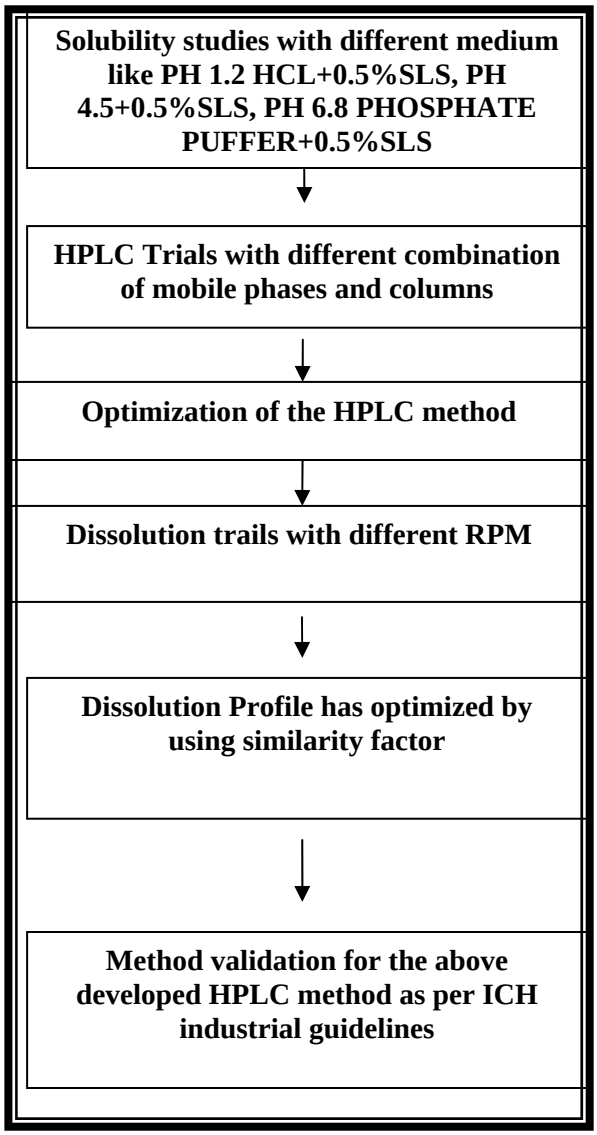
- Later several trials were done in RP-HPLC using a different combination of mobile phases and finally method was optimized.

- Various trails ware made to find out to fixing dissolution parameters like RPM, Apparatus, Dissolution volume.

- Dissolution method has been optimized by using similarity factor.

- After optimization of the HPLC method, validation of the analytical method for the developed RP-HPLC method was done in accordance with the ICH guidelines.

- The plan of work is presented in the following scheme



5. EXPERIMENTAL PART

ASSAY METHOD DEVELOPMENT

The need to save method development time and improve accuracy is forcing today's analytical chemists to look for better, faster ways to develop stability indicating methods.

Starting with HPLC columns that offer excellent reproducibility, column lifetime and sensitivity this step by step protocol can save the method development chemists time and money required to establish new method. This approach is consistent with developing process.

STEPS IN ASSAY METHOD DEVELOPMENT

1. Selection of Detector Wavelength
2. Selection and Optimization of Mobile Phase
    - pH of the buffer or pH of the Mobile Phase
    - Optimization of Mobile Phase Composition
3. Selection of Column
4. Selection of Flow Rate
5. Selection of Column Temperature
6. Selection of Diluent
7. Selection of Injection Volume
8. Establishment of System Suitability

5.1 ASSAY METHOD DEVELOPMENT

DETECTION METHOD AND SELECTION OF WAVELENGTH

Known concentrations of Nisoldipine working standard was taken and dissolved in Methanol such that the standard solution contains about 51 ppm. Placebo & blank solutions also prepared. All these solutions were scanned between 200-400 nm using UV visible spectrophotometer.

OPTIMIZATION OF MOBILE PHASE

MOBILE PHASE COMPOSITION

Trail: 1

Preparation of mobile phase

Accurately measured a volume of 1000ml of Acetonitrile (100%).Filtered and degassed for 2 mins.

Diluent: pH 3 buffer: Methanol (50:50)

Chromatographic conditions

Column	:	Peerless Basic C_{18} 100 × 4.6 mm, 1.8µ
Flow rate	:	1.0ml/min
Column oven temperature	:	30^0c
Injection volume	:	20µl
Runtime	:	10 mins

Trail: 2

Buffer Preparation:

Weighed and dissolved about 2.72 g of Potassium dihydrogen phosphate and 1.74 g of DiPotassium hydrogen phosphate in1000 mL of Milli Q water & mixed and sonicated for 10 minutes.

Preparation of mobile phase

Accurately measured a volume of 400ml of pH 6.8 Phosphate buffer and mixed with600ml of Acetonitrile (40:60) .filtered and degassed for 2 mins.

Diluent: pH 3 buffer: Methanol (50:50)

Chromatographic conditions

Column	:	Peerless Basic C_{18} 100 × 4.6 mm, 1.8µ
Flow rate	:	1.0ml/min
Column oven temperature	:	30^0c
Injection volume	:	20µl
Runtime	:	10 mins

Trail: 3

Buffer Preparation:

Weighed and dissolved about 2.72 g of Potassium dihydrogen phosphate and 1.74 g of DiPotassium hydrogen phosphate in1000 mL of Milli Q water & mixed and sonicated for 10 minutes.

Preparation of mobile phase

Accurately measured a volume of 200ml of pH 6.8 Phosphate buffer, 600ml of Acetonitrile and 200 ml of Methanol (20:60:20).filtered and degassed the solution for 2 mins.

Diluent: PH 3 buffer: Methanol (50:50)

Chromatographic conditions

Column	:	Peerless Basic C_{18} 100 × 4.6 mm, 1.8µ
Flow rate	:	1.0ml/min
Column oven temperature	:	30^0c
Injection volume	:	20µl
Runtime	:	10 mins

Trail: 4

Buffer Preparation:

Weighed and dissolved about 2.72 g of Potassium dihydrogen phosphate and 1.74 g of DiPotassium hydrogen phosphate in1000 mL of Milli Q water & mixed and sonicated for 10 minutes.

Preparation of mobile phase

Accurately measured a volume of 250ml of pH 6.8 Phosphate buffer, 500ml of Acetonitrile and 250 ml of Methanol (25:50:25).filtered and degassed the solution for 2 mins.

Diluent: PH 3 buffer: Methanol (50:50)

Chromatographic conditions

Column	:	Peerless Basic C_{18} 100 × 4.6 mm, 1.8µ
Flow rate	:	1.0ml/min
Column oven temperature	:	30^0c
Injection volume	:	20µl
Runtime	:	10 mins

Trail: 5

Buffer Preparation:

Weighed and dissolved about 2.72 g of Potassium dihydrogen phosphate and 1.74 g of DiPotassium hydrogen phosphate in1000 mL of Milli Q water & mixed and sonicated for 10 minutes.

Preparation of mobile phase

Accurately measured a volume of 300ml of pH 6.8 Phosphate buffer, 400ml of Acetonitrile and 300 ml of Methanol (30:60:30).filtered and degassed the solution for 2 mins.

Diluent: PH 3 buffer: Methanol (50:50)

Chromatographic conditions

Column	:	Peerless Basic C_{18} 100 × 4.6 mm, 1.8µ
Flow rate	:	1.0ml/min
Column oven temperature	:	30^0c
Injection volume	:	20µl
Runtime	:	10 mins

.

SOLUTION PREPARATION

Preparation of 0.02M Phosphate buffer (pH 6.8)

Weighed and dissolved about 2.72 g of Potassium dihydrogen phosphate and 1.74 g of DiPotassium hydrogen phosphate in1000 mL of Milli Q water & mixed well.

Preparation of mobile phase

Mixed pH 6.8 Phosphate buffer: methanol: acetonitrile in the ratio 30:30:40(v/v) filtered and degassed for 10 mins.

Preparation of diluent

Preparation of pH3 buffer

Weighed and dissolved about 2.72 g of Potassium dihydrogen phosphate and 1.74 g of DiPotassium hydrogen phosphate in1000 mL of Milli Q water & mixed well.

and pH was adjusted with ortho phosphoric acid.

Mixed Methanol and pH3buffer in the ratio of 50:50(v/v) respectively

Preparation of standard stock solution

Accurately weighed and transferred about 51 mg of Nisoldipine working standard into 100 mL volumetric flask, add about 40 ml MeOH, sonicated to dissolve the material completely, and add 20ml of acetonitrile and dilute to volume with MeOH and mixed.

Preparation of standard solution

Pipette 5 mL of above standard stock solution into a 50 mL volumetric flask dilute to volume with Diluents and mixed.

Test preparation

Weighed and transferred 5 tablets into a 100 ML volumetric flask added about 70 ML of diluent, sonicated for about 30 minutes with intermediate shaking, dilute to volume with diluent and mixed. The above solution was centrifuged for 10 min in centrifuge tubes at 2500 RPM; 3mL of the centrifuged sample solution was diluted to 50mL with diluent and used as the test preparation

5.2 VALIDATION OF ASSAY METHOD

VALIDATION APPROACH

Validation of analytical method was done to establish by laboratory studies, that the performance characteristics of the method meet the requirement for intended analytical application.

The following experimental design is drawn in order to prove the test method is capable to yield consistent, reliable and reproducible results within the predetermined acceptance limits

- Acceptance criteria for above validation parameters are specified in individual experimental design
- Observation and results are recorded in individual method validation data sheets.
- Summarize the finding of the method validation and draw interference
- Based on the interpretation of the results in method validation, draw the conclusion

VALIDATION PARAMETERS

The following parameters have been validated

- System suitability
- Precision
- Specificity
- Linearity
- Accuracy
- Robustness
- Ruggedness

VALIDATION PROCEDURES

1) SYSTEM SUITABILITY

The standard solution was prepared by using Nisoldipine working standard as per test method and was injected ten replicate injections into the HPLC system.

The system suitability parameters were evaluated from standard chromatograms by calculating the percentage RSD from ten replicate injections for Nisoldipine retention time and peak areas.

2) PRECISION:

The precision of an analytical method is the closeness of agreement (Degree of scattered) between series of measurements obtained from multiple samplings of the same homogeneous sample under the prescribed conditions

a) Repeatability (Method Precision)

Repeatability expresses the precision under the same operating condition over a short interval of time.

Procedure

Prepare and analysis six replicate sample preparations as per test method.

Calculated individual assay value, mean assay value, %RSD and recorded.

3) SPECIFICITY:

Specificity of analytical method is ability to measure specifically the analyte of interest without interference from blank and placebo.

a) Check for interference from placebo:

Placebo Preparation:

Weigh placebo equivalent to five intact tablets in 100ml volumetric flask, add 20ml of Acetonitrile and 50 ml of methanol and sonicated for some time, and make up the volume with methanol, take 3ml from above solution and makeup to 100ml with diluent. Inject the placebo preparation and check the interference

b) Check for interference from forced degradation studies:

Subjected the Nisoldipine tablets in following condition

1. Acid Degradation
2. Base Degradation
3. Oxidative Degradation
4. Hydrolysis Degradation
5. Photolytic Degradation

Ensure that at least one condition degradation is between 1 to 30% for each condition prepare blank accordingly

1.1 Acid Degradation:

Weigh accurately transfer five tablets into 100ml volumetric flask, add 25ml of 0.1N Hcl and sonicated with shaking to disappear the tablet completely and place in to water bath at 70^0C about 30 minutes. Add 25ml of 0.1NaoH for neutralization. Make up the volume 10ml of acetonitrile and 40ml of Methanol. From this pipette out 3ml and make up to 100ml with diluent.

1.2 Base Degradation

Weigh accurately transfer five tablets into 100ml volumetric flask, add 25ml of 0.1N Sodium Hydroxide and sonicated with shaking to disappear the tablet completely and place in to water Bath at 70^0C about30 minutes. Add 25ml of 0.1Hcl for neutralization. Make up the volume 10ml of acetonitrile and 40ml of Methanol. From this pipette out 3ml and make up to 100ml with diluent.

1.3 Oxidative Degradation

Weigh accurately transfer five tablets into 100ml volumetric flask, 25ml of 2% of Hydrogen peroxide solution and for suitable interval, place in to water Bath at 40^0C about 30 minutes and make up the volume with 10ml of acetonitrile and 40ml of Methanol.
From this pipette out 3ml and make up to 100ml with diluent.

1.4 Hydrolytic Degradation

Weigh accurately transfer five tablets into 100ml volumetric flask, add 25ml of Water and place the volumetric flask in water bath about 30 mints make up the volume with 10ml of acetonitrile and 40ml of Methanol .From this pipette out 3ml and make up to 100ml with diluent.

1.5 Photolytic Degradation

Tablet powder (covered with aluminum foil) wash exposure in photo stability chamber as per guidelines and over all illumination of NLT-1.2 million lux hour and an integrated near UV energy of NLT- 200 watt hour/Sq.m.

Weigh accurately five tablets and crush them into powder. Tablet powder of 3900mg of exposed samples was transfer into 100ml volumetric flask add 20ml of Acetonitrile and 50ml of methanol and sonicated for some time and make up the volume with methanol, from this further dilute 3ml to 100ml with diluent

Procedure:

Prepare standard and sample solution by suitable degradation method and injected into HPLC system and evaluate the peak purity.

4) LINEARITY:

The linearity of analytical method is its ability to elicit test results that are directly, of by well defined mathematical transformation, proportional to the concentration of analyte in simple within a given working range

Procedure:

A series of solutions are prepared using Nisoldipine working standard at concentration levels from 25% to 150% of target concentrations (25%, 50%, 75%, 100%, 125%, 150%) measure the peak area response of the solutions

5) ACCURACY (RECOVERY)

The accuracy of analytical method is the closeness of sample results obtained by that method to the true value. The true value is that result which would be observed in the absence of error. Accuracy may often be expressed as present recovery by assay of known, added amounts of analyte. Accuracy is a measure of the exactness of the analytical method that is true for all practical purpose.

Determine accuracy over the range 25%, 50%, 75%, 100%, 125% & 150% of the working concentration. Added calculated amount of Nisoldipine working standard or API ion placebo to attain 25%, 50%, 75%, 100%, 125% & 150%.

RECOVERY PREPARATIONS:

Level 1: (25%)

Weighed and transfer above 42.5mg of Nisoldipine into 100ml Volumetric flask, add 3780 mg of placebo add 20ml Acetonitrile and 80ml of Methanol and shake well and sonicate for some times. Pipette out 3ml of above solution and makeup the volume 100ml with diluent.

Level 2: (50%)

Weighed and transfer above 85mg of Nisoldipine into 100ml Volumetric flask, add 3780 mg of placebo add 20ml Acetonitrile and 80ml of Methanol and shake well and sonicate for some times. Pipette out 3ml of above solution and makeup the volume 100ml with diluent.

Level 3: (75%)

Weighed and transfer above 127.5mg of Nisoldipine into 100ml Volumetric flask, add 3780 mg of placebo add 20ml Acetonitrile and 80ml of Methanol and shake well and sonicate for some times. Pipette out 3ml of above solution and makeup the volume 100ml with diluent.

Level 4: (100%)

Weighed and transfer above 170mg of Nisoldipine into 100ml Volumetric flask, add 3780 mg of placebo add 20ml Acetonitrile and 80ml of Methanol and shake well and sonicate for some times. Pipette out 3ml of above solution and makeup the volume 100ml with diluent.

Level 5: (125%)

Weighed and transfer above 212.5mg of Nisoldipine into 100ml Volumetric flask, add 3780 mg of placebo add 20ml Acetonitrile and 80ml of Methanol and shake well and sonicate for some times. Pipette out 3ml of above solution and makeup the volume 100ml with diluent.

Level 6: (150%)

Weighed and transfer above 255mg of Nisoldipine into 100ml Volumetric flask, add 3780 mg of placebo add 20ml Acetonitrile and 80ml of Methanol and shake well and sonicate for some times. Pipette out 3ml of above solution and makeup the volume 100ml with diluent.

Procedure:

Prepare three preparations for each level and injected each preparation in triplicate. Calculate the amount found and percentage recovery at each level and calculate the mean percentage recovery and %RSD

6) ROBUSTNESS:

a) **Effect of variation in mobile phase composition:**

A study was conducted to determine the effect of variation in organic phase composition in mobile phase. Standard solution prepared as per the test method was injected into HPLC system using various mobile phase compositions. The system suitability parameters were evaluated and found to be within the limits.

b) Effect of variation of flow rate:

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into HPLC system using flow rate 0.9ml/min and 1.1ml/min. The system suitability parameters were evaluated and found to be within the limits for 0.9ml/min and 1.1ml/min flow.

Nisoldipine were resolved from all other peaks and the retention was compared with those obtained for mobile phase having flow rate 1.0ml/min.

c) Effect of variation in pH:

A study was conducted to determine the effect of variation in PH. Standard solution prepared as per the test method was injected into HPLC system using 6.6 and 7.

The System suitability parameters were evaluated and found to be within the limits for pH 6.6 and 7.Nisoldipine were resolved from all other peaks and retention time were comparable with those obtained for mobile phase having pH 6.8

d) Effect of variation in Temperature:

A study was conducted to determine the effect of variation in Temperature. Standard solution prepared as per the test method was injected into HPLC system at $28^{o}C$&$32^{0}C$ temperature. Similarly sample solution was chromatographed at $30^{o}C$ temperature. Nisoldipine were resolved from all other peaks and retention times were comparable with those obtained mobile phase having $30^{o}C$ temperature.

e) Filter validation

To demonstrate robustness of assay method, carry out filter validation using two different filters prepare test solution in triplicate. Centrifuge and filter different portions of the test preparation and inject into the HPLC system along with unfiltered standard. Calculate the percentage assay.

7) RUGGEDNESS:

Intermediate Precision within laboratory precision on a different day, by a different analyst, by different column by using same lot of sample as specified under repeatability. Bench top stability of mobile phase, standard and sample preparation also evaluated.

Procedure:

Repeated the procedure followed for method precision on a different day, different analyst, using different HPLC system and by different column by using same lot of sample calculate individual assay value mean assay value, %RSD, overall %RSD.

Find out the difference in the assay value of intermediate precision.

5.3 DISSOLUTION

DETECTION METHOD AND SELECTION OF WAVELENGTH

Known concentrations of Nisoldipine working standard was taken and dissolved in Methanol such that the standard solution contains about 51 ppm. Placebo & blank solutions also prepared. All these solutions were scanned between 200-400 nm using UV visible spectrophotometer.

OPTIMIZATION OF MOBILE PHASE

MOBILE PHASE COMPOSITION

Trail: 1

Buffer Preparation:

Weighed and dissolved about 2.72 g of Potassium dihydrogen phosphate and 1.74 g of DiPotassium hydrogen phosphate in1000 mL of Milli Q water & mixed and sonicated for 10 minutes.

Preparation of mobile phase

Accurately measured a volume of 300 ml of pH 6.8 Phosphate buffer and mixed with400ml of Acetonitrile 300ml of methanol (30:40:30) .filtered and degassed for 2 mins.

Diluent: dissolution medium

Chromatographic conditions

Column	:	Prontosil H C_{18} 100 × 4.6 mm, 3µ
Flow rate	:	1.5ml/min
Column oven temperature	:	Ambient
Injection volume	:	50µl
Runtime	:	10 mins

Trail: 2

Buffer Preparation:

Preparation of pH 3.0 buffer

7ml of triethylamine is mixed with 1000ml of water and ph was adjusted with ortho phosphoric acid.

Preparation of mobile phase

Accurately measured a volume of 400ml of pH 3 Phosphate buffer and mixed with600ml of Acetonitrile (40:60) .filtered and degassed for 2 mins.

Diluent: dissolution medium

Chromatographic conditions

Column	:	Prontosil H C_{18} 100 × 4.6 mm, 3µ
Flow rate	:	1.5ml/min
Column oven temperature	:	Ambient
Injection volume	:	50µl
Runtime	:	10 mins

Trail: 3

Buffer Preparation:

Preparation of pH 3.0 buffer

7ml of triethylamine is mixed with 1000ml of water and ph was adjusted with ortho phosphoric acid.

Preparation of mobile phase

Accurately measured a volume of 200ml of pH 3 Phosphate buffer and mixed with600ml of Acetonitrile ,200ml of Methanol in the ratio of(20:60:20) .filtered and degassed for 2 mins.

Diluent: dissolution medium

Chromatographic conditions

Column	:	Prontosil H C_{18} 100 × 4.6 mm, 3µ
Flow rate	:	1.5ml/min
Column oven temperature	:	Ambient
Injection volume	:	50µl
Runtime	:	10 mins

Trail: 4

Buffer Preparation:

Preparation of pH 3.0 buffer

7ml of triethylamine is mixed with 1000ml of water and ph was adjusted with ortho phosphoric acid.

Preparation of mobile phase

Accurately measured a volume of 300ml of pH 3 Phosphate buffer and mixed with400ml of Acetonitrile,300 ml of Methanol in the ratio of (30:40:30) .filtered and degassed for 2 mins.

Diluent: dissolution medium

Chromatographic conditions

Column	:	Prontosil H C_{18} 100 × 4.6 mm, 3µ
Flow rate	:	1.5ml/min
Column oven temperature	:	Ambient
Injection volume	:	50µl
Runtime	:	10 min

Solubility studies

Solubility of the drug can studied in different medium like water, 0.1HCL, pH4.5 Phosphate buffer, pH 6.8 Phosphate buffer.

Selection of dissolution parameters.

Dissolution parameters like Medium, Apparatus, RPM, Time points, Medium volume can be selected from Office of Generic Drugs. It can be optimized with different trails and using similarity factor.

Preparation of solutions

Preparation of pH 3.0 buffer

Weighed and dissolved about 2.72 g of Potassium dihydrogen phosphate and 1.74 g of DiPotassium hydrogen phosphate in1000 mL of Milli Q water & mixed well. And pH was adjusted with ortho phosphoric acid.

Preparation of mobile phase

Mixed pH3 buffer: methanol: Acetonitrile in the ratio 30:30:40(v/v) filtered and degassed for 10 mins.

Preparation of standard stock solution

Accurately weighed and transferred about 50 mg of Nisoldipine working standard into 100 ML volumetric flask, add about 70ml of methanol, sonicated to dissolve the material completely, dilute to volume with methanol and mixed.

Preparation of standard solution

Pipette 4 mL of above standard stock solution into a 50ml volumetric flask dilute to volume with dissolution medium (0.1NHCL+0.5%SLS) and mixed.

Test preparation

Weighed and transferred each individual tablet to respective dissolution vessels containing 900ML of dissolution medium (0.1NHCL+0.5%SLS), and started the run. The samples are collected at specific time point.

5.4 VALIDATION OF DISSOLUTION METHOD

VALIDATION APPROACH

Validation of analytical method was done to establish by laboratory studies, that the performance characteristics of the method meet the requirement for intended analytical application.

The following experimental design is drawn in order to prove the test method is capable to yield consistent, reliable and reproducible results within the predetermined acceptance limits

- Acceptance criteria for above validation parameters are specified in individual experimental design
- Observation and results are recorded in individual method validation data sheets.
- Summarize the finding of the method validation and draw interference
- Based on the interpretation of the results in method validation, draw the conclusion

VALIDATION PARAMETERS

The following parameters have been validated

- System suitability
- Precision
- Specificity
- Linearity
- Accuracy
- Robustness
- Ruggedness
- Sink condition

VALIDATION PROCEDURES

1) SYSTEM SUITABILITY

The standard solution was prepared by using Nisoldipine working standard as per test method and was injected ten times into the HPLC system .The system suitability parameters were evaluated from standard chromatograms by calculating the percentage RSD from ten replicate injections for Nisoldipine retention time and peak areas.

2) PRECISION:

The precision of an analytical method is the closeness of agreement (Degree of scattered) between series of measurements obtained from multiple samplings of the same homogeneous sample under the prescribed conditions

A) Repeatability (Method Precision)

Repeatability expresses the precision under the same operating condition over a short interval of time.

Procedure

Prepare and analysis six replicate sample preparations as per method. Calculated individual %Dissolution value, %RSD and 95% confidence interval and recorded.

3) SPECIFICITY:

Specificity of analytical method is ability to measure specifically the analyte of interest without interference from blank and placebo.

a) Check for interference from placebo:

Placebo Preparation:

Weigh placebo equivalent to one tablet, transfer in to dissolution vessel and add 900 ml of dissolution medium (0.1NHCL+0.5%SLS), and started the run. The samples are collected at specific time point.(24^{th} hour).Inject the sample and check the interference

4) LINEARITY:

The linearity of analytical method is its ability to elicit test results that are directly, of by well defined mathematical transformation, proportional to the concentration of analyte in simple within a given working range

Procedure:

A series of solutions are prepared using Nisoldipine working standard at concentration levels from 25% to 150% of target concentrations (25%, 50%, 75%, 100%, 125%, 150%) measure the peak area response of the solutions

5) ACCURACY (RECOVERY)

The accuracy of analytical method is the closeness of sample results obtained by that method to the true value. The true value is that result which would be observed in the absence of error. Accuracy may often be expressed as present recovery by assay of known, added amounts of analyte. Accuracy is a measure of the exactness of the analytical method that is true for all practical purpose.

Determine accuracy over the range 25%, 50%, 75%, 100%, 125% & 150% of the working concentration. Added calculated amount of Nisoldipine working standard or API ion placebo to attain 25%, 50%, 75%, 100%, 125% & 150%.

RECOVERY PREPARATIONS:

Level 1: (25%)

Weighed and transfer about 8.5mg of Nisoldipine and add 756 mg of placebo, transfer in to dissolution vessel and add 900 ml of dissolution medium (0.1NHCL+0.5%SLS), and started the run. The samples are collected at specific time point (24^{th} hour).

Level 2: (50%)

Weighed and transfer about 17mg of Nisoldipine and add 756 mg of placebo, transfer in to dissolution vessel and add 900 ml of dissolution medium (0.1NHCL+0.5%SLS), and started the run. The samples are collected at specific time point (24^{th} hour).

Level 3: (75%)

Weighed and transfer about 25.5mg of Nisoldipine and add 756 mg of placebo, transfer in to dissolution vessel and add 900 ml of dissolution medium (0.1NHCL+0.5%SLS), and started the run. The samples are collected at specific time point (24^{th} hour).

Level 4: (100%)

Weighed and transfer about 34mg of Nisoldipine and add 756 mg of placebo, transfer in to dissolution vessel and add 900 ml of dissolution medium (0.1NHCL+0.5%SLS), and started the run. The samples are collected at specific time point (24^{th} hour).

Level 5: (125%)

Weighed and transfer about 42.5mg of Nisoldipine and add 756 mg of placebo, transfer in to dissolution vessel and add 900 ml of dissolution medium (0.1NHCL+0.5%SLS), and started the run. The samples are collected at specific time point (24^{th} hour).

Level 6: (150%)

Weighed and transfer about 51mg of Nisoldipine and add 756 mg of placebo, transfer in to dissolution vessel and add 900 ml of dissolution medium (0.1NHCL+0.5%SLS), and started the run. The samples are collected at specific time point (24^{th} hour).

Procedure:

Prepare three preparations for each level and injected each preparation in triplicate. Calculate the amount found and percentage recovery at each level and calculate the mean percentage recovery and %RSD.

6) ROBUSTNESS:

a) Effect of variation of flow rate:

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into HPLC system using flow rate 1.4ml/min and 1.6ml/min. The system suitability parameters were evaluated and found to be within the limits

for 1.4ml/min and 1.6ml/min flow. Nisoldipine were resolved from all other peaks and the retention was compared with those obtained for mobile phase having flow rate 1.5ml/min.

b) Effect of variation in pH:

A study was conducted to determine the effect of variation in pH. Standard solution prepared as per the test method was injected into HPLC system using 2.8 and 3.2The System suitability parameters were evaluated and found to be within the limits for pH 2.8 and 3.2. Nisoldipine were resolved from all other peaks and retention time were comparable with those obtained for mobile phase having pH 3

c) Filter validation

To demonstrate robustness of dissolution method, carry out filter validation using two different filters. Prepare test solution in triplicate. Centrifuge and filter different portions of the test preparation and inject into the HPLC system along with unfiltered standard. Calculate the percentage dissolution.

7) RUGGEDNESS:

Intermediate Precision within laboratory precision on a different day, by a different analyst, by different column by using same lot of sample as specified under repeatability.

Procedure:

Repeated the procedure followed for method precision on a different day, different analyst, using different HPLC system and by different column by using same lot of sample calculate individual assay value mean assay value, %RSD, overall %RSD.

Find out the difference in the assay value of intermediate precision.

8) SINK CONDITION

Perform dissolution on Nisoldipine drug substance in dissolution medium by adding 102mg of Nisoldipine to dissolution vessel at room temperature with 150 rpm. Calculate the % dissolution of Nisoldipine.

6 RESULTS AND DISSCUSION

6.1 ASSAY

Optimization of chromatographic conditions

Suitable selection of the method depends upon the nature of the sample, its molecular weight and solubility. Hence the drugs selected in the present study polar in nature, RP-HPLC method was selected because of its simplicity.

For the determination of wavelength for the estimation of Nisoldipine, UV spectrum was recorded in the range of 200-400 nm. After receiving the spectrum of standard, sample, placebo, wavelength of detection method was selected as 333nm. (Fig no 1.1-1.4)

The optimization of HPLC parameters was done by investigating the influence of the mobile phase composition while stationary phase, detection wavelength, injection volume, flow rate and column oven temperature were set constant.

In the case of Trail 1 acetonitrile was selected as mobile phase but resulted in no elution of analyte. In the case of Trail 2 pH6.8 Phosphate buffer: acetonitrile was selected as mobile phase at a ratio of 50:50. Potassium phosphate buffer is selected basing on literature studies and it's finalized. pH of the buffer can be selected based on the pKa value of the drug. Nisoldipine has 7.2 as a pKa. So the pH of the buffer can be fixed near to that pKa value. But resulted in poor tailing and poor peak shape (Figure2.1). Hence pH6.8 Phosphate buffer: Acetonitrile: methanol was selected as a mobile phase at a ratio of 20:60:20 and chromatograms were recorded in Trail 3. While using methanol in the mobile phase, peak shape was good. But the retention time was too short and theoretical plate count also very less (Figure 2.2). So in Trail 4 and 5 the above mobile phase ratio was adjusted in to 25:50:25&30:40:30 respectively. Finally in the Trail 5 the peaks with good symmetry, optimized retention time, tailing factor were observed (figure 2.3, 2.4) and therefore this was selected as the mobile phase condition for this method the column thermostat was maintained at 30˚C. Initially injection column was stabilized with the initial mobile phase concentration for 60 min.

Many trials on composition of buffer and organic phase Results are as follows

Table No 6.1.1

Composition of Mobile phase.	**Retention Time**	**Tailing factor**	**Theoretical plates**
ACN	-	-	-
PH6.8 PHOSPHATE BUFFER:ACN 40:60	6.4	2.7	2906
PH6.8 PHOSPHATE BUFFER: ACN:MeOH (20:60:20)	2.6	1.6	6399
PH6.8 PHOSPHATE BUFFER: ACN:MeOH (25:50:25)	2.8	1.6	6773
PH6.8 PHOSPHATE BUFFER: ACN:MeOH (30:40:30)	5.2	1.5	12173

SELECTION OF COLUMN

Keeping the mobile phase ratio 30:40:30 pH 6.8 phosphate buffer: Acetonitrile: Methanol, the chromatograms were recorded with C18 Column (peerless Basic (100 X 4.6mm) 1.8µm. At this column, the peaks were sharp. So this column was kept constant for the analysis (also tried with different particle size of stationary phase like 5µ, 3µ.)3µ column also produce good peak symmetry and retention time. For the measurement of Peak purity we can go with 1.8 µ column.

Table 6.1.2

Column	Retention Time(min)	Area	Tailing factor	Theoretical plates
PRONTOSIL C_{18}(150*4.6)5µ	6.5	897275	1.3	7052
NUCLEODUR C_{18}(100*4.6)3µ	4.5	896747	1.5	7506
PEERLESS BasicC_{18}(100*4.6) 1.8 µ	5.2	904747	1.5	12186

EFFECT OF FLOW RATE:

Keeping the mobile phase ratio 30:40:30 pH 6.8 phosphate buffer: Acetonitrile: Methanol, the chromatograms were recorded at a flow rate of 1.0ml/min. At this flow rate, the peaks were sharp. So 1.0ml/min was kept constant for the analysis (flow rate 0.8ml/min 1.2ml/min, up to 1.5ml/min were also tried, but did not give any satisfactory results).

Results Found Are As Follows

Table 6.1.3

Flow rate mL /min	Retention time	Tailing factor	Theoretical Plates
0.8	6.2	1.5	13365
1.0	5.2	1.5	12190
1.2	4.1	1.5	11373
1.5	3.3	1.5	9890

EFFECT OF COLUMN OVEN TEMPERATURE

Keeping the mobile phase ratio 30:40:30 pH 6.8 phosphate buffer: Acetonitrile: Methanol, the chromatograms were recorded at an oven temperature at 30^0C. At this flow condition, the peaks were sharp. So 30^0C was kept constant for the analysis (column oven temperature ambient, 35^0C, 40^0C were also tried, but did not give any satisfactory results).

Results Found Are As Follows

Table 6.1.4

Temp °C	Retention time	Tailing factor	Theoretical plates
ambient	5	1.6	12531
30°c	5.2	1.5	12197
35^0 C	4.6	1.5	11949
40°c	4.3	1.4	11162

ADJUSTING THE INJECTION VOLUME

Keeping the mobile phase ratio 30:40:30 pH 6.8 phosphate buffer: Acetonitrile: Methanol, the chromatograms were recorded as injection volume 20. At this condition, the peaks were sharp. So 20 µL was kept constant for the analysis (injection volume 40 µL, 50 µL, 80 µL were also tried)

Table 6.1.5

Injection vol (µL)	Retention Time	Area	Tailing Factor	Theoretical Plates
20	5.2	901396	1.5	12237
40	4.9	1664761	1.5	12457
50	5	2078280	1.6	12473
80	5	3317006	1.6	12280

QUANTITATIVE DETERMINATION OF THE DRUG

Preparation of pH 6.8 Phosphate buffer

Weighed and dissolved about 2.72 g of Potassium dihydrogen phosphate and 1.74 g of DiPotassium hydrogen phosphate in1000 mL of Milli Q water & mixed well.

Preparation of mobile phase

Mixed pH 6.8 Phosphate buffer: methanol: acetonitrile in the ratio 30:30:40(v/v) filtered and degassed for 10 mins.

Preparation of diluent

Preparation of pH3 Phosphate buffer

Weighed and dissolved about 2.72 g of Potassium dihydrogen phosphate and 1.74 g of DiPotassium hydrogen phosphate in1000 mL of Milli Q water & mixed well. And pH was adjusted with ortho phosphoric acid.

Mixed Methanol and pH3buffer in the ratio of 50:50(v/v) respectively

Preparation of standard stock solution

Accurately weighed and transferred about 51 mg of Nisoldipine working standard into 100 mL volumetric flask, add about 40 ml MeOH, sonicated to dissolve the material completely, and add 20ml of acetonitrile and dilute to volume with MeOH and mixed.

Preparation of standard solution

Pipette 5 mL of above standard stock solution into a 50 mL volumetric flask dilute to volume with Diluents and mixed.

Test preparation

Weighed and transferred 5 tablets into a 100 ML volumetric flask added about 70 ML of diluent, sonicated for about 30 minutes with intermediate shaking, dilute to volume with diluent and mixed. The above solution was centrifuged for 10 min in centrifuge tubes at 2500 RPM; 3mL of the centrifuged sample solution was diluted to 50mL with diluent and used as the test preparation

Fixed Chromatographic system

1. Liquid chromatograph equipped with a 333 nm UV detector.
2. Column: 100 x 4.6 mm column that contains 1.8 µm packing of octadecyl silane chemically bonded to porous silica or ceramic micro particles [peerless Basic(100 X 4.6mm)1.8µm]
3. Column temperature : 30°c
4. Flow rate : 1.0 mL/min
5. Injection volume : 20 µl

6. Run time : 9 min

System Suitability

Inject 20 µL portion of diluent (single injection) and standard solution (five replicate injections) into the chromatograph record the chromatograms and measure the responses for the major peak.

The USP tailing factor for Nisoldipine peak should not be more than 2.0

CALCULATION

$$\text{Amount of Nisoldipine Present (\% label claim)} = \frac{AxWsx5x100x100 \times Px100}{Bx100x50xNx3 \times Lx100}$$

A=Peak area of Nisoldipine in test preparation.

B=Average peak area of Nisoldipine in standard preparation.

Ws=Weight of Nisoldipine working standard taken, in mg

P=Potency of Nisoldipine m working standard taken

L=Label claim of Nisoldipine in mg, per tablet

N=Number of tablet taken

6.2 METHOD VALIDATION- ASSAY

SYSTEM SUITABILITY AND SYSTEM PRECISION

1. SYSTEM PRECISION

Inject ten replicate injections of Nisoldipine standard preparation into HPLC system and calculate relative deviation of Nisoldipine peak area.

Table 6.2.1

Injection no.	Nisoldipine peak area
1	932986
2	933694
3	933202
4	932596
5	932928
6	933031
7	932978
8	932223
9	933270
10	933167
Average	933007
% RSD	0.15

SYSTEM SUITABILITY

Prepare system suitability solution as per the developed method and inject into HPLC system

Table 6.2.2

System Suitability Parameters	Observed value	Acceptance limit
Tailing	1.5	NMT 2.0
plate count	11577	NLT 3500
% RSD of Retention time	0.45	NMT 2.0%

Acceptance Criteria

1) The % RSD for the peak area from ten replicate injections of Nisoldipine should be NMT 2%
2) The %RSD for the retention time of Nisoldipine from ten replicate injection of Standard Solution should not be more than 2%
3) The number of theoretical plates (N) for the Nisoldipine peak is NLT 3500
4) The Tailing factor (T) for the Nisoldipine peak is NMT 2.0

Observation

1) The % RSD for the peak area from ten replicate injections of Nisoldipine is 0.15
2) The %RSD for the retention time of Nisoldipine from ten replicate injection of Standard Solution should 0.45
3) The number of theoretical plates (N) for the Nisoldipine peak is 11577
4) The Tailing factor (T) for the Nisoldipine peak is 1.5

Conclusion

It passes the system suitability

2. SPECIFICITY

PLACEBO INTERFERENCE

Perform assay in triplicate on placebo with equivalent concentration to that of the test concentration in triplicate and evaluate the interference for each of the placebo preparations.

Table 6.2.3

Sample no.	% interference
1	0
2	0
3	0

Acceptance criteria

No Interference from Placebo

Conclusion

% interference from placebo was found to be within the acceptance criteria

INTERFERENCE FROM DEGRADANTS

Table 6.2.4

Sample name	Purity angle		Purity threshold		Purity flag
	Drug substance	Drug product	Drug substance	Drug product	
Unstressed	0.107	0.103	0.273	0.239	No
Stressed with 0.1N HCL on a water bath at 70 °c for 30 min	0.067	0.095	0.236	0.238	No
Stressed with 0.1N NaOH on a water bath at 75 °c for 3hrs	0.082	0.065	0.249	0.237	No
Stressed with 2% H_2O_2solution by keeping on bench top for 30 min	0.099	0.147	0.236	0.228	No
Stressed with water on water bath at 75°c for 8 hrs	0.091	0.084	0.238	0.237	No
Stressed with UV for about 54 hrs	0.068	0.068	0.235	0.237	No
Stressed with visible light for about 288hrs	0.070	0.080	0.226	0.232	No

Acceptance criteria

Purity angle should be less than purity threshold.

Nisoldipine peaks should not have any flag in purity results table

Conclusion

% interference from degradants was found to be within the acceptance criteria

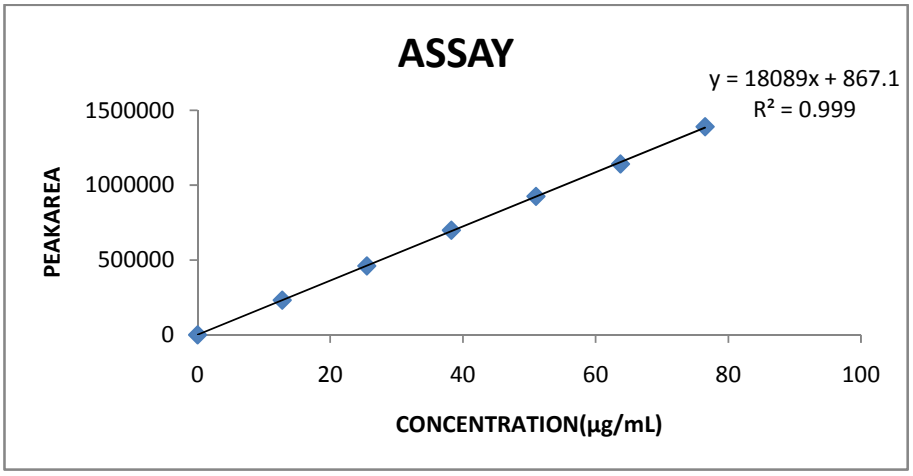
3. LINEARITY OF METHOD

To demonstrate the linearity of assay method, inject in duplicate five standard solutions with concentration equivalent to 25 % to 150% target concentration of standard. Plot a graph to concentration versus average peak area.

Table 6.2.5

S. No.	Conc (µg/mL)	Average area
1	12.75	231773
2	25.5	460356
3	38.25	699126
4	51	925418
5	63.75	1142144
6	76.5	1390715
Coefficient correlation = 0.9998		

Chart No: 1



Acceptance Criteria

Coefficient of correlation shall be NLT 0.999

4. PRECISION

Repeatability

Determine the precision of the developed method by assaying six samples of homogenous blend prepared by mixing placebo and Nisoldipine raw material

Table 6.2.6

Test no	Area	Average area	% Assay
1	934043	933844	100.4
	933646		
2	934420	934496	100.5
	934572		
3	932840	932821	100.3
	932802		
4	932447	932786	100.3
	933026		
5	931972	931972	100.2
	931972		
6	932084	931972	100.3
	932374		
Average		932982	100.3
% RSD		0.10	0.10

Acceptance criteria

1) The Average Assay should be not less than 95%.
2) The relative standard deviation of assay should not be more than 2.0%.

Observation

1) The Average assay is 100.3
2) The relative standard deviation of assay is 0.10

Conclusion: The results obtained are well within the acceptance criteria. Therefore method is precise.

5. ACCURACY

To validate, the assay method can accurately quantify Nisoldipine within the excipients, proportionally spiking various amounts of Nisoldipine raw material and the placebo at various concentrations ranging from 25 % to 150 %.

Table 6.2.7

Sample no	Spike level %	mg added	mg found	% recovery	avg. % recovery	% RSD
1	25	8.5	8.5	100	99.6	0.7
			8.5	100		
			8.4	98.8		
2	50	17	17	100	99.6	0.7
			16.8	98.8		
			17	100		
3	75	25.5	25.4	99.6	99.7	0.2
			25.4	99.6		
			25	100		
4	100	34	34	100	99.9	0.2
			33.9	99.7		
			34	100		
5	125	42.5	42.5	100	99.8	0.3
			42.5	100		
			42.3	99.5		
6	150	51	50.9	99.8	99.7	0.1
			50.8	99.6		
			50.9	99.8		

Acceptance Criteria

The average recovery of Nisoldipine at each spike level should not be less than 95% and not more than 105%.

Conclusion

The % recovery at each level is found to be within the limits.

Hence the method is accurate in the specified range.

6. RUGGEDNESS

a. Analyst to analyst variability

To demonstrate intermediate precision of assay method, conduct analyst to analyst variability by two analysts. Analyst 2 shall perform precision of test method on different day preparing three samples of Nisoldipine tablet blend, prepared by mixing placebo and Nisoldipine raw material as per the composition.

Table 6.2.8

System suitability	Observed values		Acceptance criteria
	Analyst-1	Analyst-2	
Tailing factor	1.5	1.5	NMT 2
plate count	11495	11483	NLT 3500

Table 6.2.9

Sample no	Assay of Nisoldipine as % of labeled amount	
	Analyst-1	Analyst-2
1	100.3	99.7
2	99.9	98.9
3	100.2	100.2
Average	100.1	99.6
%RSD	0.20	0.70
Over all % RSD	0.45	

Acceptance criteria

The all individual assays of Nisoldipine should be within 95% to 105%

Relative standard deviation of % assay results should not be more than 2.0% by both the analysts.

The overall RSD for 12 values shall be NMT 2.0 %.

b. System to system variability

To demonstrate the ruggedness of assay method, carryout system to system variability on two HPLC systems using same column under similar conditions. Assay three different preparations prepared by mixing Nisoldipine raw material and the placebo as per composition given in the formula.

Table 6.2.10

System suitability	Observed values		Acceptance criteria
	System- 1 (shimadzu)	System- 2 (shimadzu)	
Tailing factor	1.5	1.5	NMT 2
USP plate count	11496	11519	NLT 3500

Table 6.2.11

Sample no	**Assay of Nisoldipine as % of labeled amount**	
	System-1	**System-2**
1	99.6	99.7
2	99.4	99.6
3	99.3	99.2
Average	99.4	99.5
%RSD	0.20	0.30
Overall % RSD	0.25	

Acceptance criteria

The all individual assays of Nisoldipine should be within 95% to 105%.

Relative standard deviation of % assay results should not be more than 2.0% by both the analysts

The overall RSD for 6 values shall be NMT 2.0 %.

c. Column to column variability

To demonstrate the ruggedness of assay method, carryout column to column variability using the columns of the same manufacturer or different manufacturer on the same HPLC system under similar conditions. Assay three different preparations prepared by mixing Nisoldipine raw material and the placebo as per composition given in the formula

Table 6.2.12

System suitability	**Observed values**		**Acceptance Criteria**
	Column-1 (Peerless)	**Column-2 (Hypersil BDS)**	
Tailing factor	1.5	1.5	NMT 2
USP plate count	11558	11603	NLT 3500

Table 6.2.13

Sample no.	**Assay of Nisoldipine as % of labeled amount**	
	column-1	**column-2**
1	99.6	99.9
2	99.9	100.1
3	100.2	100.3
Average	99.9	100.1
%RSD	0.30	0.20
Overall % RSD	0.25	

Acceptance criteria

The all individual assays of Nisoldipine should be within 95% to 105%.

Relative standard deviation of % assay results should not be more than 2.0% by both the columns.

The overall RSD for 6 values shall be NMT 2.0 %.

d. Bench top stability of mobile phase

Prepare the mobile phase as per the developed method and keep it on bench top with well closed condition. Prepare the system suitability solution, standard solution and test solutions prepared by mixing placebo and Nisoldipine raw material as per formula. Inject the system suitability solutions, standard solutions and the test solutions at initial, 1 day and 2 days. Evaluate the system suitability parameters and % assay.

Table 6.2.14

System suitability parameters	**Observed values**			**Acceptance criteria**
	Initial	**1 day**	**2 day**	
Tailing factor	1.5	1.5	1.5	NMT 2.0
USP plate count	11594	11523	11454	NLT 3500

Table 6.2.15

Approx. time in days	**% assay**	**Difference from initial**
Initial	100	NA
1 day	99.3	0.7
2 day	99.3	0.7

Acceptance criteria

The % assay result should not differ from initial value by more than 2.0%.

All system suitability parameters shall meet the requirements.

e. Bench top stability of test & standard preparations of Nisoldipine

Perform the assay of Nisoldipine tablets prepared by mixing placebo and Nisoldipine as per the formula and prepare the test in duplicate on tablets. Keep the standard, test preparations on bench top, and analyse at 1-day intervals up to 2-days against a freshly prepared standard each time.

Table 6.2.16

Time in days	% assay of Std preparation	Difference	% assay of test preparation		Difference	
			Test-1	Test-2	Test-1	Test-2
Initial	100	NA	99.5	99.7	NA	NA
Day 1	99.8	0.2	99.5	99.3	0.0	0.4
Day 2	98.9	1.3	98.5	98.8	1.0	1.1

Acceptance criteria

The % assay of Nisoldipine in standard and test preparation should not differ by more than 2.0 % from initial value.

f. Refrigerator stability of Nisoldipine std & test preparations

Perform the assay of Nisoldipine tablets prepared by mixing placebo and Nisoldipine as per the formula and prepare the test in duplicate on tablets. Keep the standard, test preparations in refrigerator, and analyse at 1-day intervals up to 2-days against a freshly prepared standard each time

Table 6.2.17

Time in days	% assay of Std preparation	Difference	% assay of test preparation		Difference	
			Test-1	Test-2	Test-1	Test-2
Initial	100	NA	99.8	99.9	NA	NA
1	99.8	0.2	99.0	99.2	0.8	0.7
2	98.9	1.1	98.7	98.5	1.1	1.3

Acceptance Criteria: The % assay of Nisoldipine in standard and test preparation should not differ by more than 2.0 % from initial value.

Conclusion:

The Reproducibility of the method comes under within the limits.

7. ROBUSTNESS

a. Effect of variation in mobile phase composition

To demonstrate the robustness, check the system suitability parameters by injecting system suitability solution and standard preparation, by using two mobile phases, one containing 10% increase in composition of organic solvent and other containing 10% decrease in the composition of organic solvent. Evaluate the system suitability parameters and % assay

Table 6.2.18

Suitability parameters	**Organic phase ratio**			**Acceptance criteria**
	90% org.	**100 % org.**	**110% org.**	
Tailing factor	1.5	1.5	1.5	NMT 2
plate count	11019	11212	11045	NLT 3500

If the acceptance criterion fails, narrow the organic phase composition range and report the organic phase composition range at which acceptance criteria passes.

Table 6.2.19

Mobile phase composition	**% assay**		**Average % assay**
	Trail-1	**Trail-2**	
25:50:25	99.4	99.0	99.2
30:40:30	100.0	99.0	99.5
35:30:35	100.3	99.8	100

Acceptance criteria

All system suitability parameters shall meet the requirements.

The average %assay values should not differ by more than 2.0 when compared with that of the test values.

b. Effect of variation in flow rate

To demonstrate the robustness of method, check the system suitability parameters by injecting system suitability solution and standard preparation, into the HPLC system with 0.9 mL/min and 1.1mL/min. evaluate the system suitability parameters and % assay.

Table 6.2.20

Suitability parameters	**Flow rate mL/min**			**Acceptance criteria**
	0.9mL/min	**1.0mL/min**	**1.1mL/min**	
Tailing factor	1.1	1.0	1.2	NMT 2
USP plate count	11031	11223	11256	NLT 3500

If the system suitability fails, narrow the flow rate range and report the flow rate range at which system suitability passes.

Table 6.2.21

Flow rate in mL/min	**% assay**		**Average % assay**
	Trail-1	**Trail-2**	
0.9	96.6	96.6	99.6
1.0	98.8	98.9	98.8
1.1	100.2	99.9	100.1

Acceptance criteria

All system suitability parameters shall meet the requirements.

The average %assay values should not differ by more than 2.0% when compared with that of the test values.

c. Effect of variation in pH

To demonstrate the robustness of method, check the system suitability parameters by injecting standard preparation, into the HPLC system at ±0.2 pH .Evaluate the system suitability parameters and % assay.

Table 6.2.22

Suitability parameters	**Observed values at pH**			**Acceptance criteria**
	6.6	**6.8**	**7.0**	
Tailing factor	1.5	1.5	1.5	NMT 2
Usp plate count	11154	11212	11145	NLT 3500

If the system suitability fails, narrow the pH range and report the pH range at which system suitability passes.

Table 6.2.23

pH	% assay
6.6	99.3
6.8	100.1
7.0	100.4

Acceptance criteria

All system suitability parameters shall meet the requirements.

The average %assay values should not differ by more than 2.0 when compared with that of the test values.

d. Effect of variation in column temperature

To demonstrate the robustness of method, check the system suitability parameters by injecting standard preparation into the HPLC system at 40°c temperature, 35°c and at 45 °c . Evaluate the system suitability parameters and % assay.

Table 6.2.24

Suitability parameters	Observed values at column temp.			Acceptance criteria
	28°c	30°c	32°c	
Tailing factor	1.5	1.5	1.4	NMT 2
USP plate count	11019	11423	11405	NLT 3500

Table 6.2.25

Column oven temperature	% assay
28 °c	99.3
30 °c	97.9
32 °c	97.5

Acceptance criteria

All system suitability parameters shall meet the requirements.

The average %assay values should not differ by more than 2.0 when compared with that of the test values.

e. Filter validation

To demonstrate robustness of assay method, carry out filter validation using two different filters prepare test solution in triplicate. Centrifuge and filter different portions of the test preparation and inject into the HPLC system along with unfiltered standard. Calculate the percentage assay.

Table 6.2.26

Filter description	**filters**	
	1	**2**
Manufactured by	Advanced micro devices pvt ltd	Advanced micro devices pvt ltd
Lot no	SYNN0602MNX104	NB417178
Size	0.45um	0.45um

Table 6.2.27

Centrifuged	**Samples filtered through-filter**		**Samples filtered through-filter**	
% assay	**% assay**	**Difference**	**% assay**	**Difference**
99.8	99.6	0.2	99.4	0.4
99.6	99.4	0.2	99.1	0.5
99.7	99.6	0.1	99.5	0.2
99.8	99.3	0.5	99.6	0.2

Acceptance Criteria

The difference of %assay result from centrifuged sample to filtered samples should be not more than 2.0.

6.3 DISSOLUTION

Optimization of chromatographic conditions

Suitable selection of the method depends upon the nature of the sample, its molecular weight and solubility. Hence the drugs selected in the present study polar in nature, RP-HPLC method was selected because of its simplicity.

For the determination of wavelength for the estimation of Nisoldipine, UV spectrum was recorded in the range of 200-400 nm. After receiving the spectrum of standard, sample, placebo, wavelength of detection method was selected as 333nm.

The optimization of HPLC parameters was done by investigating the influence of the mobile phase composition while stationary phase, detection wavelength, injection volume , flow rate and column oven temperature were set constant.

In the case of Trail 1 pH6.8 Phosphate buffer: acetonitrile: methanol was selected as mobile phase at a ratio of 30:40:30 but resulted in no elution. Figure (7.1). In the case of Trail 2 pH3 (near to log p value of Nisoldipine) buffer: acetonitrile was selected as mobile phase at a ratio of 50:50 but resulted in poor elution Figure (7.2). Hence pH3 buffer: Acetonitrile: methanol was selected as a mobile phase at a ratio of 20:60:20 and chromatograms were recorded in Trail 3. While using methanol in the mobile phase, peak shape was good. But the retention time was too long and theoretical plate count also very less (Figure7.3). So in Trail 4 the above mobile phase ratio was adjusted in to 30:40:30. In the Trail 4 the peaks with good symmetry, optimized retention time, tailing factor were observed (figure7.4) and therefore this was selected as the mobile phase condition for this method the column thermostat was maintained at ambient temperature. Initially injection column was stabilized with the initial mobile phase concentration for 60 min.

Many trials on composition of buffer and organic phase were done to decide the ultimate composition of mobile phase. Results found are as follows

Table no 6.3.1

Composition of mobile phase.	**Retention time**	**Tailing factor**	**Theoretical plates**
PH6.8 PHOSPHATEBUFFER :ACN:Methanol(30:40: 30)	No elution		
PH6.8 PHOSPHATE BUFFER: ACN(50:50)	9.2	1.1	10536
PH6.8 PHOSPHATE BUFFER: ACN:MeOH(20:60:20)	4.2	1.4	5567
PH6.8 PHOSPHATE BUFFER: ACN:MeOH(30:40:30)	3.1	1.1	5989

SELECTION OF COLUMN

Keeping the mobile phase ratio 30:40:30 pH 3 buffer: Acetonitrile: Methanol, the chromatograms were recorded with C18 Column (Prontosil H (100 X 4.6mm) 3µm. At this column, the peaks were sharp. So this column was kept constant for the analysis (also tried with different particle size of stationary phase like 5µ, 1.8µ.)1.8µ column also produce good peak symmetry and retention time. Due to efficacy of column (life time) 3 µ was selected.

Table 6.3.2

Column	**Retention time**	**Area**	**Tailing factor**	**Theoretical plates**
Prontosil C_{18} H (100*4.6mm)3µ	3.1	1177890	1.0	5969
Peerless Basic C_{18}(100*4.6mm)1.8µ	3.4	1179252	1.2	1304
Develosil (150*4.6mm) 5µ	4.2	1163862	1.5	1854

EFFECT OF FLOW RATE:

Keeping the mobile phase ratio 30:40:30 pH 3 buffer: Acetonitrile: Methanol, the chromatograms were recorded at a flow rate of 1.5ml/min. At this flow rate, the peaks were sharp and short retention time. So 1.5ml/min was kept constant for the analysis (flow rate 0.8ml/min 1ml/min, up to 1.2ml/min were also tried, but did not give any satisfactory results).

.Results Found Are As Follows

Table no 6.3.3

Flow rate mL /min	Peak shape	Retention time	Tailing factor	Theoretical plates
0.8	-	-	-	-
1.0	Good	4.3	1.0	6019
1.2	Good	3.6	1.0	5705
1.5	Excellent	3.1	1.0	5930

EFFECT OF COLUMN OVEN TEMPERATURE

Keeping the mobile phase ratio 30:40:30 pH 3 buffer: Acetonitrile: Methanol, the chromatograms were recorded at an oven temperature at ambient (due to addition of SLS). At this temperature, the peaks were sharp. So Ambient temperature was kept constant for the analysis (column oven temperature 30^0C, 350C, 40^0C were also tried, but there is no effective changes in the retention time).Results Found Are As Follows.

Table no 6.3.4

Temp °c	Peak shape	Retention time	Tailing factor	Theoretical plates
ambient	Excellent	3.1	1.0	5992
30°c	Good	3.1	1.2	4663
35°C	Good	2.9	1.2	4069
40°c	Good	2.8	1.4	4982

SELECTION OF DISSOLUTION PROFILE

Solubility studies

Conduct solubility studies (saturation solubility) of the drug substance in dissolution media having pH between 1 to 8

Table no 6.3.5

S.NO	Media	Solubility (mg/mL)
1	Purified water+0.5%SLS-25^{0}C	0.091
2	Purified water+0.5%SLS-37^{0}C	0.115
3	0.1N HCL+0.5%SLS-25^{0}C	0.069
4	0.1N HCL+0.5%SLS-37^{0}C	0.079
5	PH 6.8 Phosphate buffer+0.5%Tween 80-25^{0}C	0.074
6	PH 6.8 Phosphate buffer+0.5%Tween 80-37^{0}C	0.072

Dissolution medium 0.1N HCL+0.5%SLS can be selected as drug solubility studies and information from office of drug generic.

SELECTION OF RPM

Table no 6.3.6

TIME(Hr/Min)	% Drug release at 50 Rpm	% Drug release at 50 Rpm
2	7.4	8.2
6	30.8	31.7
12	68.1	74.8
20	94.5	101.6
24	101.6	102.1

From the above result, maximum amount of %drug release can be obtained in 50 RPM itself. So 50 RPM has selected.

Dissolution Profile Comparison by Using Similarity Factor

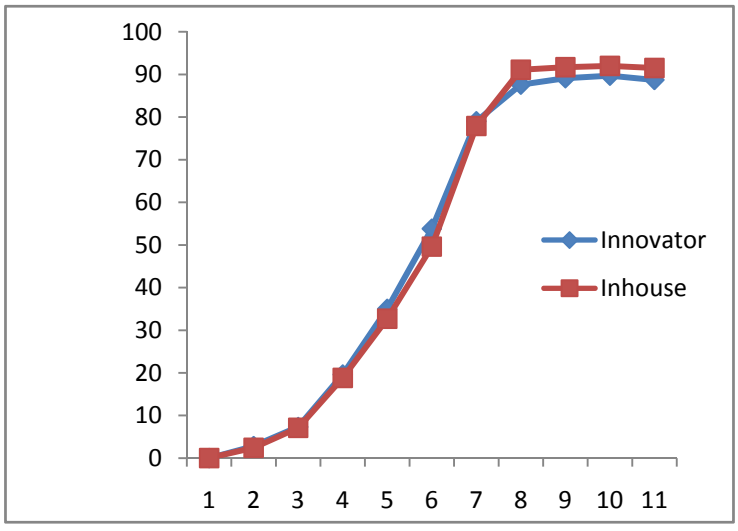
Among several methods investigated for dissolution profile comparison, f2 is the simplest. More and Flanner proposed a model independent mathematical approach to compare the dissolution profile using two factors, f1 (difference factor) and f2 (similarity factor).it can be shown below.

a) COMPARATIVE DRUG RELEASE PROFILES OF INNOVATOR & INHOUSE IN USP Type II, 50 RPM, pH 1.2 + 0.5% SLS, Sinkers

Table no 6.3.7(A)

Time (h)	Innovator	In house
0	0	0
1	2.8	2.4
2	7.3	7.1
4	19.6	18.8
6	35	32.7
8	53.8	49.6
12	79	77.9
16	87.6	91.1
18	89.1	91.7
20	89.7	92
24	88.7	91.5
f2 (>50)		**80.0**
f1 (<15)		**4.4**

Chart No:2

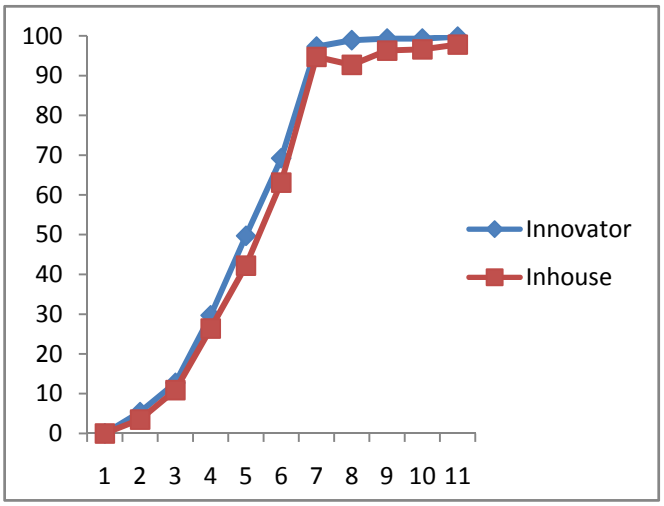


COMPARATIVE DRUG RELEASE PROFILES OF INNOVATOR & INHOUSE IN USP Type II, 50 RPM, pH 4.5 + 0.5% SLS, Sinkers

Table no 6.3.7(b)

Time (h)	Innovator	In house
0	0	0
1	5.4	3.5
2	12.7	10.9
4	29.7	26.4
6	49.7	42.2
8	69.2	63.1
12	97.3	94.7
16	98.9	92.7
18	99.3	96.3
20	99.3	96.6
24	99.8	97.8
f2 (>50)		67.1
f1 (<15)		8.8

Chart No:3

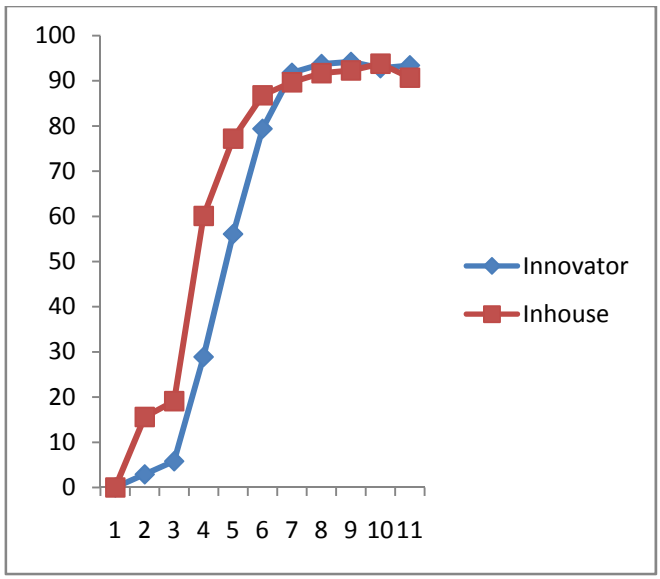


COMPARATIVE DRUG RELEASE PROFILES OF INNOVATOR & INHOUSE IN USP Type II, 50 RPM, pH 6.8 BUFFER + 0.5% SLS, Sinkers

Table no 6.3.7(c)

Time (h)	Innovator	In house
0	0	0
1	2.9	15.6
2	5.8	19.1
4	28.9	60.1
6	56.1	77.2
8	79.4	86.8
12	91.8	89.7
16	93.7	91.7
18	94.2	92.3
20	92.8	93.8
24	93.4	90.7
f2 (>50)		36.0
f1 (<15)		49.5

Chart No:4

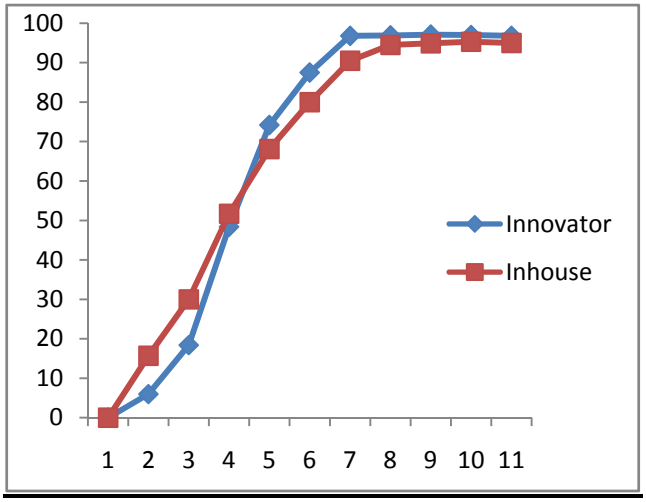


COMPARATIVE DRUG RELEASE PROFILES OF INNOVATOR & INHOUSE IN USP Type II, 50 RPM, pH 7.5 BUFFER + 0.5% SLS, Sinkers

Table no 6.3.7(d)

Time (h)	Innovator	In house
0	0	0
1	6	15.7
2	18.4	30
4	48.4	51.7
6	74.2	68.1
8	87.5	80
12	96.8	90.5
16	96.9	94.5
18	97.1	94.9
20	97	95.3
24	96.8	95
f2 (>50)		**54.3**
f1 (<15)		**16.3**

Chart No:5

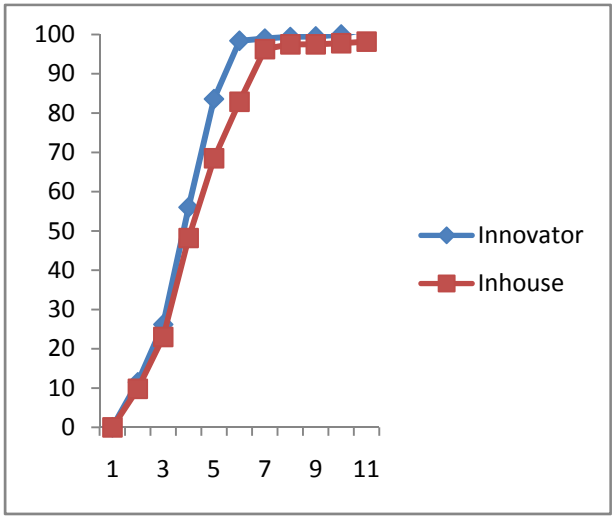


COMPARATIVE DRUG RELEASE PROFILES OF INNOVATOR &INHOUSE IN USP Type II, 50 RPM, Purified Water + 0.5%SLS, Sinkers

Table no 6.3.7(e)

Time (h)	Innovator	In house
0	0	0
1	11.5	9.8
2	26.2	23
4	56	48.2
6	83.6	68.5
8	98.4	82.9
12	99	96.3
16	99.4	97.5
18	99.5	97.5
20	100	97.8
24	101.4	98.2
f2 (>50)		**59.0**
f1 (<15)		**14.7**

Chart No:6



OPTIMIZED METHOD FOR DISSOLUTION

Chromatographic system

1. Liquid chromatograph equipped with a 333 nm UV detector.
2. **Column**: 100 x 4.6 mm column that contains 3 µm packing of octadecyl silane chemically bonded to porous silica or ceramic micro particles (Prontosil H -100 x 4.6 mm)
3. **Column temperature** **:** Ambient
4. **Flow rate** **:**1.5 mL/min
5. **Injection volume** **:**50 µl
6. **Run time** **:** 5 mins

Dissolution parameters

1. Dissolution medium **:** 0.1 N HCL+0.5%SLS

2. Dissolution apparatus **:** USP 2 Paddle

3. RPM **:** 50 RPM

4. Dissolution volume **:** 900 ML

5. Time points **:** 4, 8, 16, 24

System Suitability

Inject 20 µL portion of diluents (single injection) and standard solution (five replicate injections) into the chromatograph record the chromatograms and measure the responses for the major peak.

The USP tailing factor for Nisoldipine peak should not be more than 2.0

The column efficiency for Nisoldipine peak should be not less than 3500 theoretical plates.

The % RSD for the areas of Nisoldipine peak obtained from five replicate injections of standard solution should be not more than 2.0

FORMULA

CALCULATION

$$\text{Quantity of Nisoldipine dissolved in } n^{th} \text{ time interval (D)} = \frac{A \times WS \times 4 \times 900 \times P \times 100}{B \times 100 \times 50 \times L \times 100}$$

CALCULATION OF CORRECTION FACTOR $F(n-1) = (D(n-1)/900) \times 10$

CORRECTED RESULTS

n^{th} time interval = $D(n-1)+F1+F2\ldots\ldots+F(n-1)$

Where, A = peak area B = average standard area

WS = weight of working standard p = potency

L = label claim

SIMILARITY FACTOR (f_2)

$$f_2 = 50 \text{ X log } \{[1+(1/n)\Sigma_{t=1} n (R_t - T_t)^2]^{-0.5} \text{ X } 100$$

DIFFERENCE FACTOR (f_1)

$$f_1 = \{ [\Sigma_{t=1} n (R_t - T_t)]/ [\Sigma_{t=1} n R_t]\} \text{ X } 100$$

Where, n = number of time points

R_t = Dissolution value of reference batch at time t.

T_t = Dissolution value of test batch at time t

6.4 METHOD VALIDATION

1. SYSTEM PRECISION

Inject ten replicate injections of Nisoldipine standard preparation into HPLC system and calculate relative deviation of Nisoldipine peak area.

Table 6.4.1

INJECTION NO.	NISOLDIPINE PEAK AREA
1	1183809
2	1184552
3	1171419
4	1160887
5	1180634
6	1189539
7	1178208
8	1173146
9	1181353
10	1173940
Average	1171749
% RSD	0.38

Acceptance Criteria: The % RSD for ten replicate injections should be NMT 1%

SYSTEM SUITABILITY

Prepare system suitability solution as per the developed method and inject into HPLC system

Table 6.4.2

System suitability parameters	Observed value	Acceptance limit
USP tailing factor	1.4	NMT 2.0
USP plate count	5689	NLT 3500
% RSD	0.71	NMT 2.0%

Acceptance criteria

1) The % RSD for the peak area from ten replicate injections of Nisoldipine should be NMT 2%
2) The %RSD for the retention time of Nisoldipine from ten replicate injection of

   Standard Solution should not be more than 2%

3) The number of theoretical plates (N) for the Nisoldipine peak is NLT 3500
4) The Tailing factor (T) for the Nisoldipine peak is NMT 2.0

Observation

1) The % RSD for the peak area from ten replicate injections of Nisoldipine is 0.38
2) The %RSD for the retention time of Nisoldipine from ten replicate injection of

   Standard Solution should 0.20

3) The number of theoretical plates (N) for the Nisoldipine peak is 5689
4) The Tailing factor (T) for the Nisoldipine peak is 1.4

Conclusion: It passes the system suitability

2. SPECIFICITY

PLACEBO INTERFERENCE (selectivity)

Perform dissolution in triplicate on placebo with equivalent concentration to that of the test concentration and evaluate the interference for each of the placebo preparations.

Table 6.4.3

Sample no	% interference
1	0
2	0
3	0

Acceptance criteria

No Interference from Placebo shall be observed at the retention time of main peak.

Conclusion

% interference from placebo was found to be within the acceptance criteria

3. PRECISION

REPEATIBILITY

Determine the precision of the method by performing dissolution on six samples of homogenous blend prepared by mixing placebo and Nisoldipine raw material.

Table 6.4.4

Sample no.	Area	Avg. area	% Dissolution
1	1182521	1183207	99.1
	1183892		
2	1189305	1186164	99.5
	1183022		
3	1179357	1176398	98.5
	1173439		
4	1187774	1185910	99.3
	1184045		
5	1187488	1189771	100.1
	1192053		
6	1187556	1189565	100.1
	1191573		
Average		1181923	99
% RSD		0.4	0.5

Acceptance criteria

1) The Average Assay should be not less than 95%.
2) The relative standard deviation of assay should not be more than 2.0%.

Observation: The Average % Dissolution is 99

The relative standard deviation of assay is 0.50

Conclusion : The results obtained are well within the acceptance criteria. Therefore method is Precise.

4. ACCURACY

To validate, the dissolution method can accurately quantify Nisoldipine with in the excipients, proportionally spiking various amounts of Nisoldipine raw material and the placebo at various concentrations ranging from 25 % to 150 %.

Table 6.4.5

Sample no	Spike level %	mg added	mg found	% recovery	avg. % recovery	%RSD
1	25	8.6	8.6	100	99.4	1.0
			8.5	98.3		
			8.6	100		
2	50	17	17.1	100.5	100.1	0.3
			17	100		
			17	100		
3	75	25.6	25.4	99.2	99.7	0.5
			25.6	100.0		
			25.6	100.0		
4	100	34	34	100	100	0.0
			34	100		
			34	100		
5	125	42.6	42.6	100	99.8	0.3
			42.4	99.5		
			42.6	100		
6	150	51	51.1	100.1	99.9	0.2
			50.9	99.8		
			51	100		

Acceptance Criteria

The average recovery of Nisoldipine at each spike level should not be less than 95%

Conclusion

The % recovery at each level is found to be within the limits.

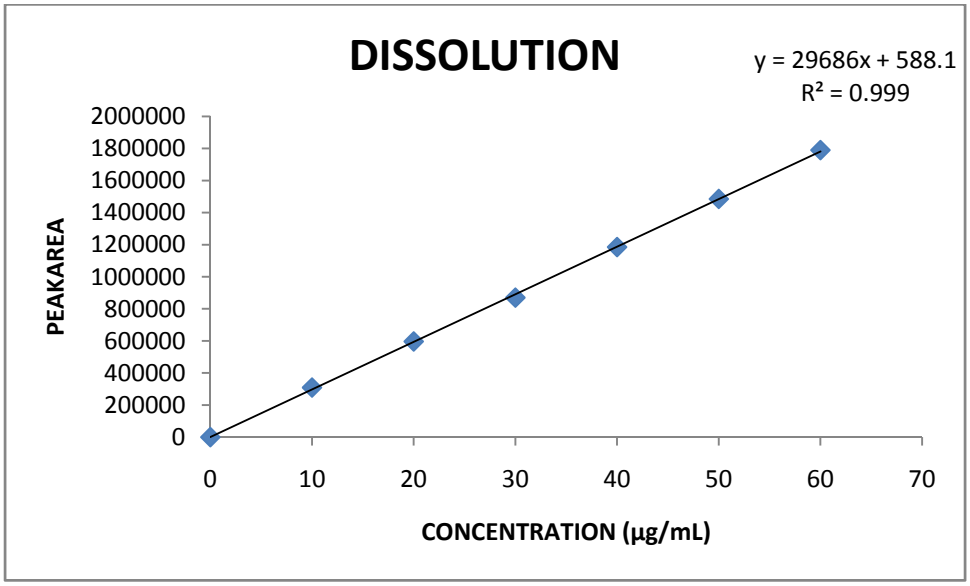
Hence the method is accurate in the specified range

5. LINEARITY OF TEST METHOD

To demonstrate the linearity of dissolution method, inject in duplicate five standard solutions with concentration equivalent to 25 % to 150% target concentration of standard. Plot a graph to concentration versus average peak area. Table 6.4.6

S. No.	Conc (µg/mL)	Average area
1	10	309421
2	20	596984
3	30	870176
4	40	1185569
5	50	1485569
6	60	1790353
Coefficient correlation = 0.9997		

Chart No: 7



Acceptance criteria

Coefficient of correlation shall be NLT 0.999

6. RUGGEDNESS

a. System to System Variability

To demonstrate the ruggedness of dissolution method, carryout system to system variability on two different dissolution systems of different make and perform dissolution.

Table 6.4.7

System suitability	**Observed values**		**Acceptance criteria**
	System-1 (Labindia)	**System-2 (Electrolab)**	
Tailing factor	1.1	1.1	NMT 2
Theoretical plate count	5655	5589	NLT3500

Table 6.4.8

Sample no	**% Dissolution**	
	System-1	**System-2**
1	99.7	99.5
2	99.8	99.8
3	99.7	99.8
Average	99.7	99.7
%RSD	0.10	0.10
Overall % RSD	0.10	

Acceptance criteria

The average dissolution should be not less than 95.0%.The relative standard deviation of dissolution should be not more than 2.0% on both the systems.

The overall RSD for 6 values shall be NMT 2.0 %.

b. Column to Column Variability

To demonstrate the ruggedness of dissolution method, carryout column to column variability by two different columns

Table 6.4.9

System suitability	**Observed values**		**Acceptance Criteria**
	Column-1 (Prontosil H)	**Column-2 (Peerless basic)**	
Tailing factor	1.2	1.1	NMT 2
Theoretical plate count	5785	5258	NLT 3500

Table 6.4.10

Sample no	**% Dissolution**	
	Column-1	**Column-2**
1	99.8	99.8
2	99.7	99.8
3	99.8	99.9
Average	99.8	99.8
%RSD	0.13	0.05
Overall % RSD	0.10	

Acceptance criteria

The average dissolution should be not less than 95%.

The relative standard deviation of dissolution should not be more than 2.0% on both the columns.

The overall RSD for 6 values shall be NMT 2.0 %.

c. Analyst to analyst variability

To demonstrate the ruggedness of dissolution method, carryout analyst to analyst variability by two different analyst

Table 6.4.11

System suitability	**Observed values**		**Acceptance criteria**
	Analyst-1	**Analyst-2**	
Tailing factor	1.1	1.1	NMT 2
Theoretical plate count	5489	5658	NLT3500

Table 6.4.12

Sample no	**% dissolution**	
	Analyst -1	**Analyst -2**
1	99.0	98.9
2	98.7	99.8
3	99.7	98.8
Average	99.2	99.4
%RSD	0.46	0.49
Overall % RSD	0.47	

Acceptance criteria

The average dissolution should be not less than 95.0%.

The relative standard deviation of dissolution should be not more than 2.0%.

The overall RSD for 6 values shall be NMT 2.0 %.

7. ROBUSTNESS

a. EFFECT OF VARIATION IN FLOW RATE

To demonstrate the robustness of method, check the system suitability parameters by injecting system suitability solution and standard preparation, into the HPLC system with 0.8 mL/min and 1.2mL/min.evaluate the system suitability parameters and % dissolution.

Table 6.4.13

Suitability parameters	**Flow rate mL/min**		**Acceptance criteria**
	1.4	**1.6**	
Tailing factor	1.1	1.0	NMT 2
Theoretical plate count	5798	5705	NLT 3500
% RSD	1.5	0.87	NMT 2.0%

If the system suitability fails, narrow the flow rate range and report the flow rate range at which system suitability passes.

Table 6.4.14

Flow rate in mL/min	**% dissolution**		**Avg.% dissolution**
	Trail-1	**Trail-2**	
1.4	99.9	100.2	100.0
1.6	99.4	99.4	99.4

Acceptance criteria

All system suitability parameters shall meet the requirements.

The average % dissolution values should not differ by more than 2.0%.

b. EFFECT OF VARIATION IN pH

To demonstrate the robustness of method, check the system suitability parameters by injecting standard preparation, into the HPLC system at +/- 0.2 pH .evaluate the system suitability parameters and % dissolution.

Table 6.4.15

Suitability parameters	**Observed values at pH**			**Acceptance criteria**
	2.8	**3.0**	**3.2**	
Tailing factor	1.1	1.2	1.1	NMT 2
Theoretical plate count	5023	5569	5456	NLT 3500
% RSD	0.5	0.51	0.79	NMT 2.0%

If the system suitability fails, narrow the pH range and report the pH range at which system suitability passes.

Table 6.4.16

pH	**% dissolution**		**Avg.% dissolution**
	Trail-1	**Trail-2**	
2.8	102.3	101.9	102.1
3.0	99.9	100.0	99.9
3.2	99.1	98.8	98.9

Acceptance criteria

All system suitability parameters shall meet the requirements. The average % dissolution values should not differ by more than 2.0 when compared with that of the test values.

C. FILTER VALIDATION

To demonstrate robustness of dissolution method, carry out filter validation using two different filters. Prepare test solution in triplicate. Centrifuge and filter different portions of the test preparation and inject into the HPLC system along with unfiltered standard. Calculate the percentage dissolution.

Table 6.4.17

Filter description	**Filters**	
	Filter 1	**Filter 2**
Manufactured by	Advanced micro devices pvt ltd	Advanced micro devices pvt ltd
Lot no	SYNN0602MNX104	NB417178
Size	0.45um	0.45um

Table 6.4.18

% dissolution (labindia)	**Samples filtered through-filter 1**		**Samples filtered through-filter 2**	
	% dissolution	**Difference**	**% dissolution**	**Difference**
98.4	97.4	1.0	97.6	0.8
98.3	97.3	1.0	97.5	0.8
98.3	97.4	0.9	97.0	1.3

Acceptance Criteria

The difference of % dissolution result between centrifuged sample and filtered samples should be not more than 2.0

9. SINK CONDITION

Perform dissolution on Nisoldipine drug substance in dissolution medium by adding 102mg of Nisoldipine to dissolution vessel at room temperature with 150 rpm. Calculate the % dissolution of Nisoldipine.

Table 6.4.19

Sample no	**'mg of Nisoldipine added'**	**'mg of Nisoldipine found'**	**%dissolution**
01	102	82.5	80.88
02	102.2	82.3	80.68
average	102.1	82.4	100

Acceptance criteria

The amount of Nisoldipine dissolved shall not be less than 60mg.

SELECTION OF WAVELENGTH

FIG-1.1

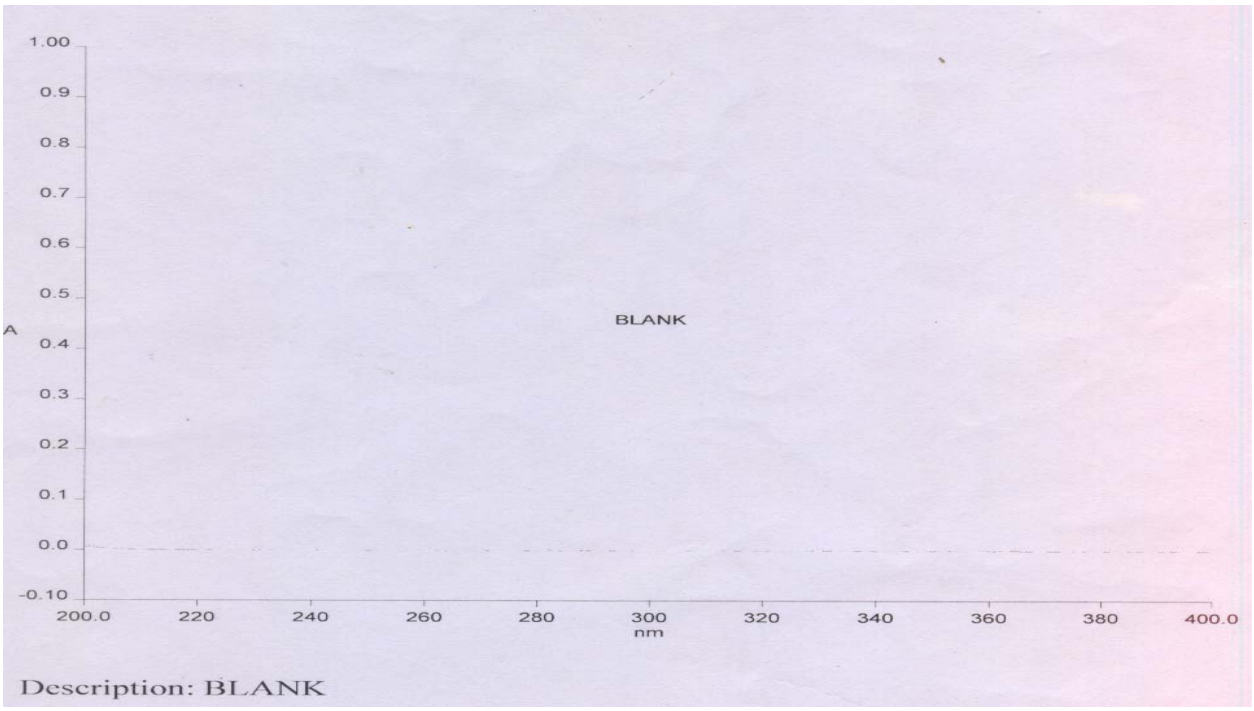


FIG-1.2

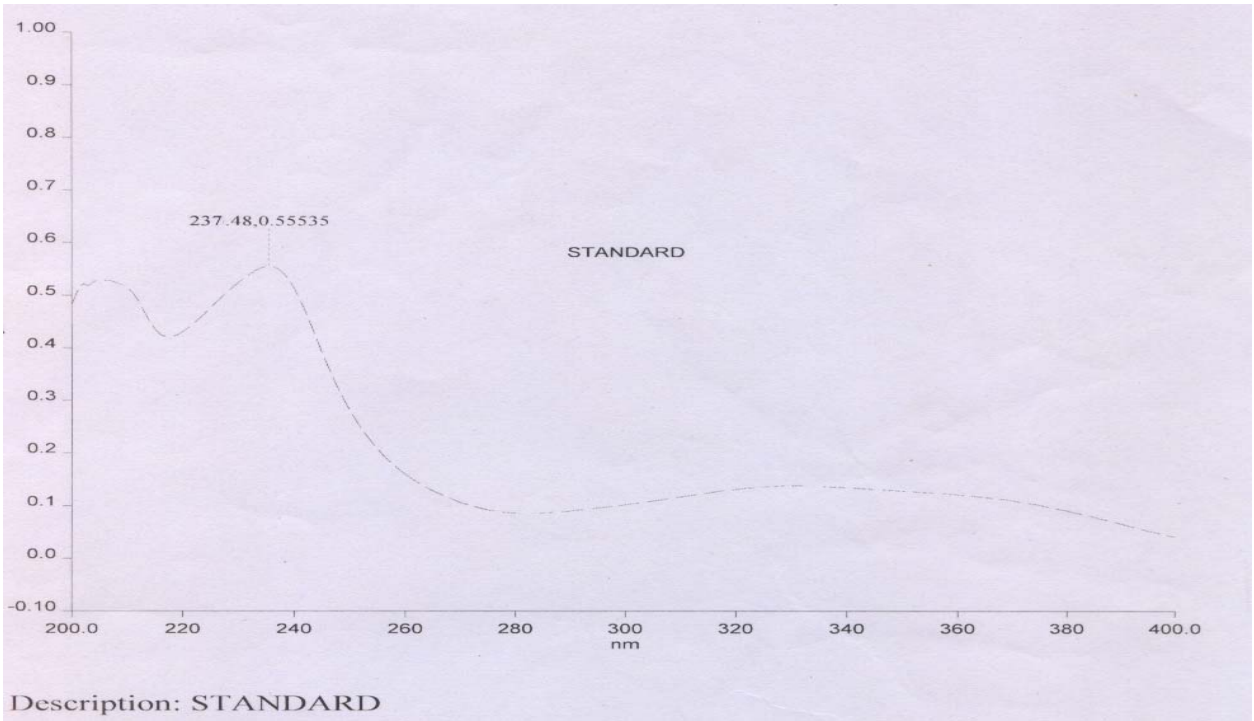


FIG-1.3

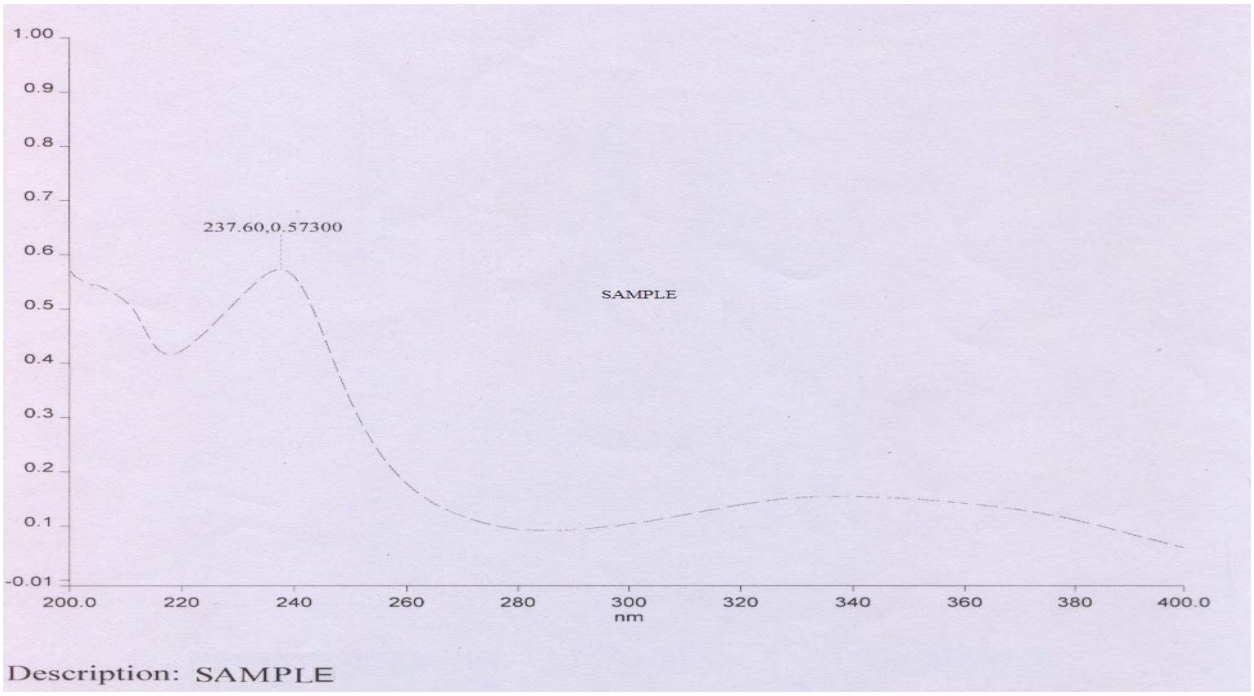
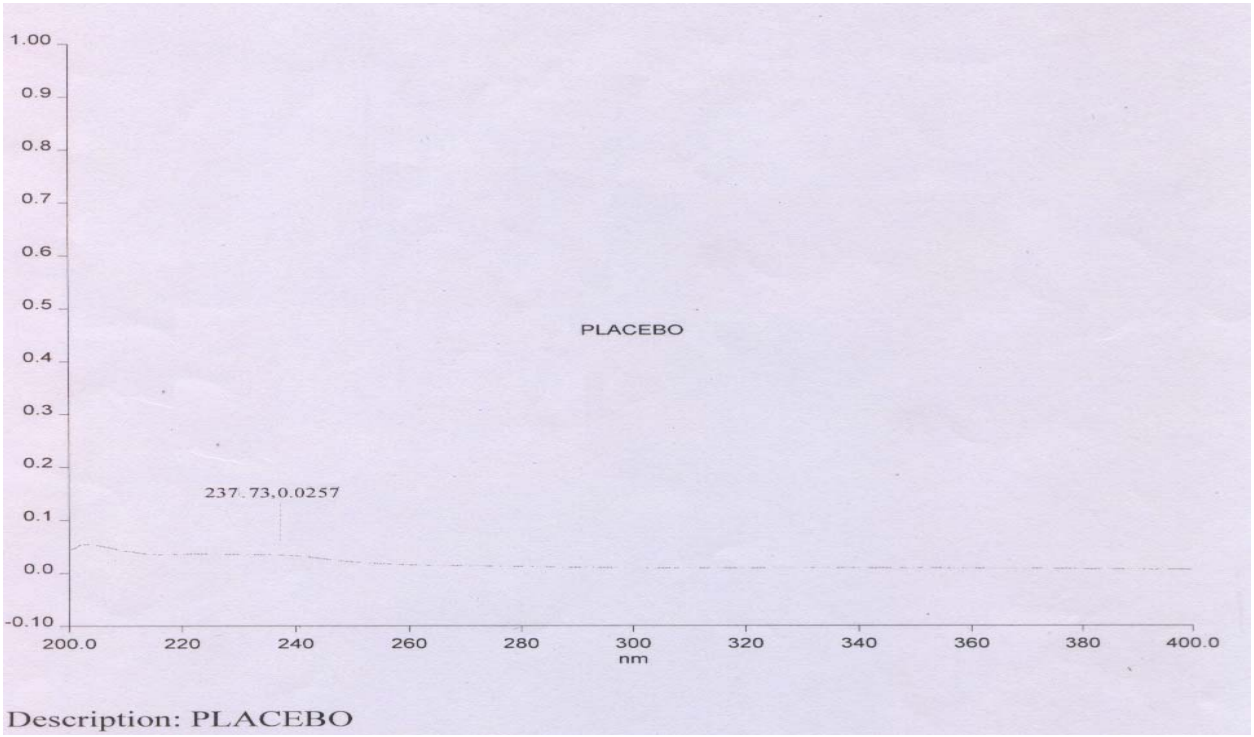
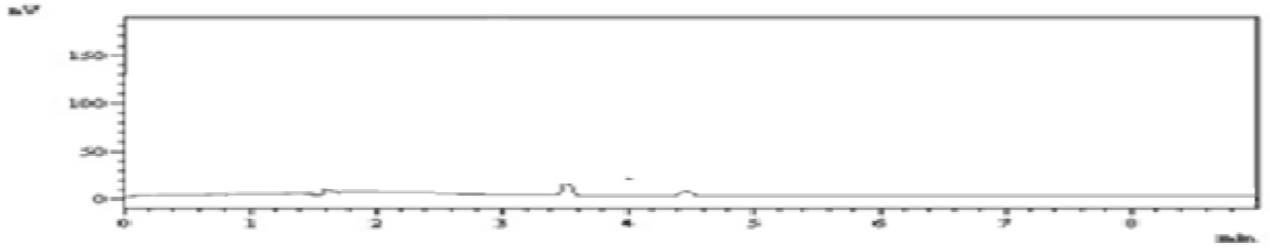
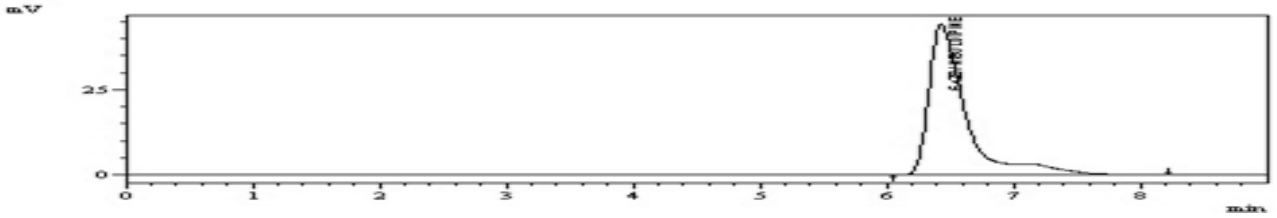

1.00
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
-0.01
237.60,0.57300
SAMPLE
200.0
220
240
260
280
300
320
340
360
380
400.0
nm
Description: SAMPLE

FIG-1.4

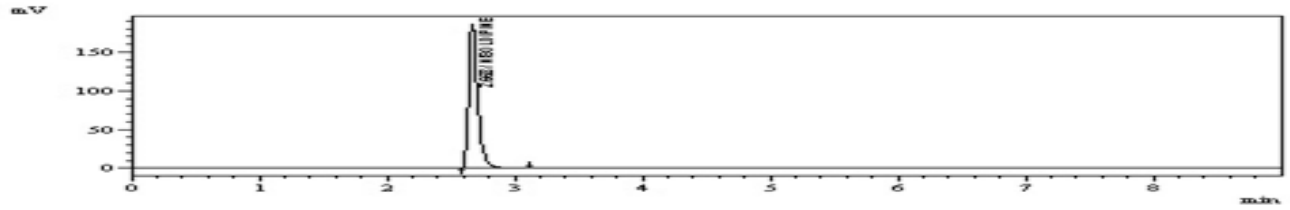

1.00
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.10
PLACEBO
237.73,0.0257
200.0
220
240
260
280
300
320
340
360
380
400.0
nm
Description: PLACEBO



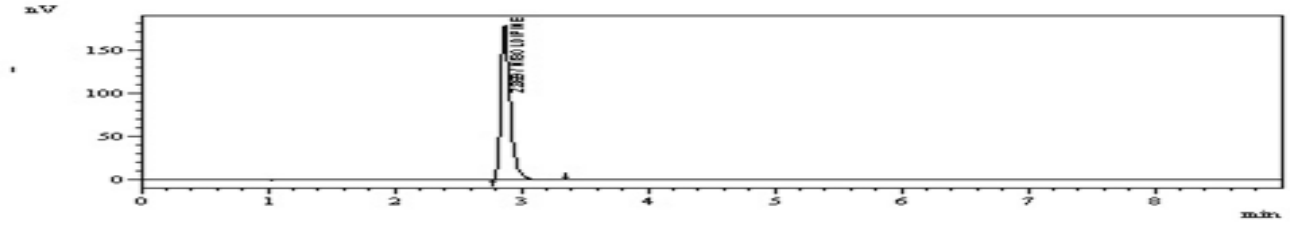
TRAIL 1 (FIG NO: 2.1)



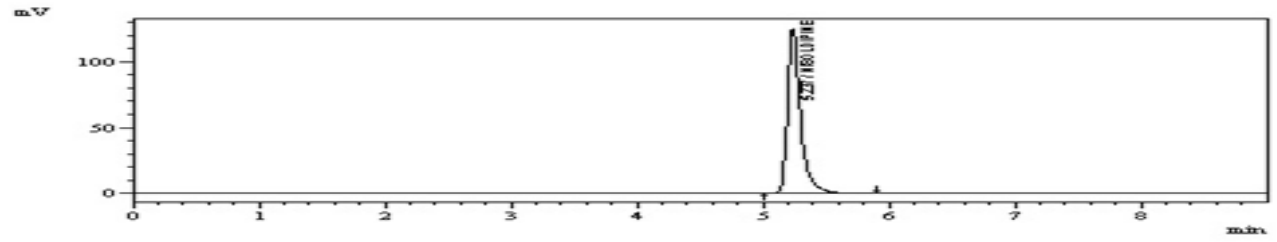
TRAIL-2 (FIG NO: 2.2)



TRAIL-3 (FIG NO: 2.3)



TRAIL-4 (FIG NO: 2.4)



TRAIL-5 (FIG NO: 2.5)

VALIDATION CHROMATOGRAMS-ASSAY

SYSTEM PRECISION

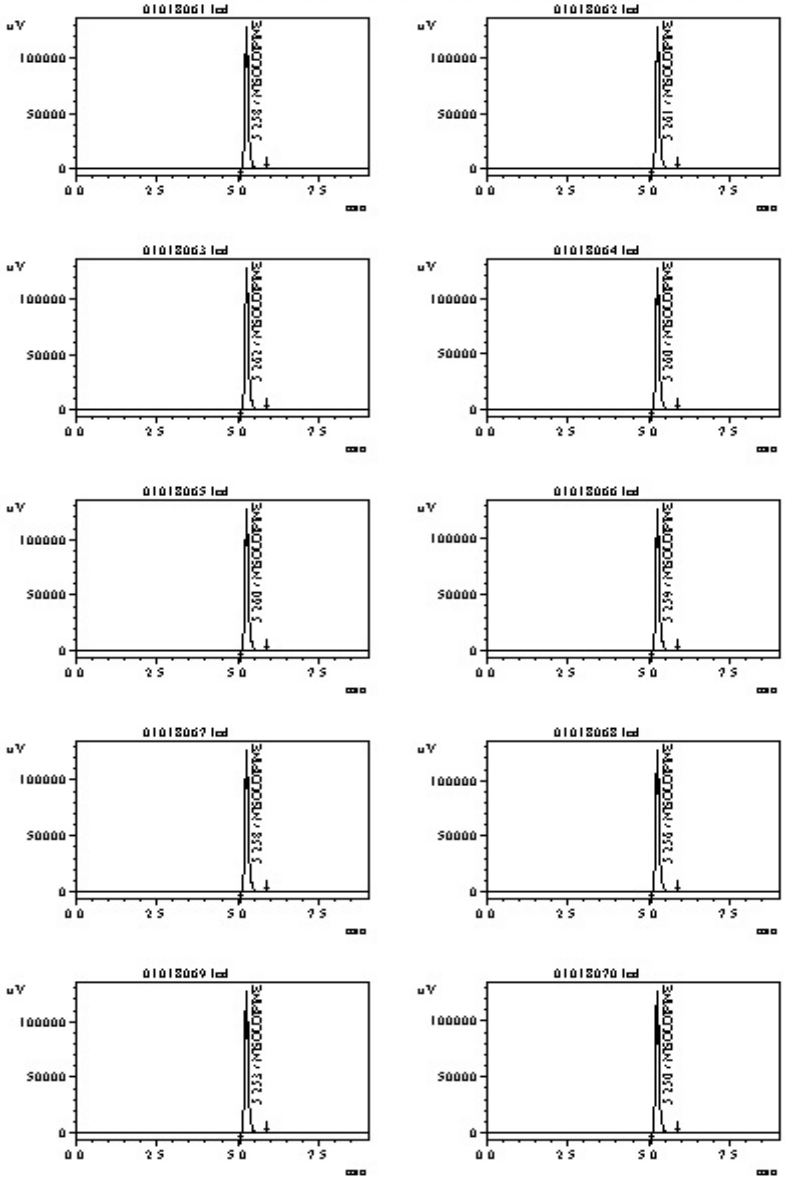


FIG NO: 3

SYSTEM SUITABILITY

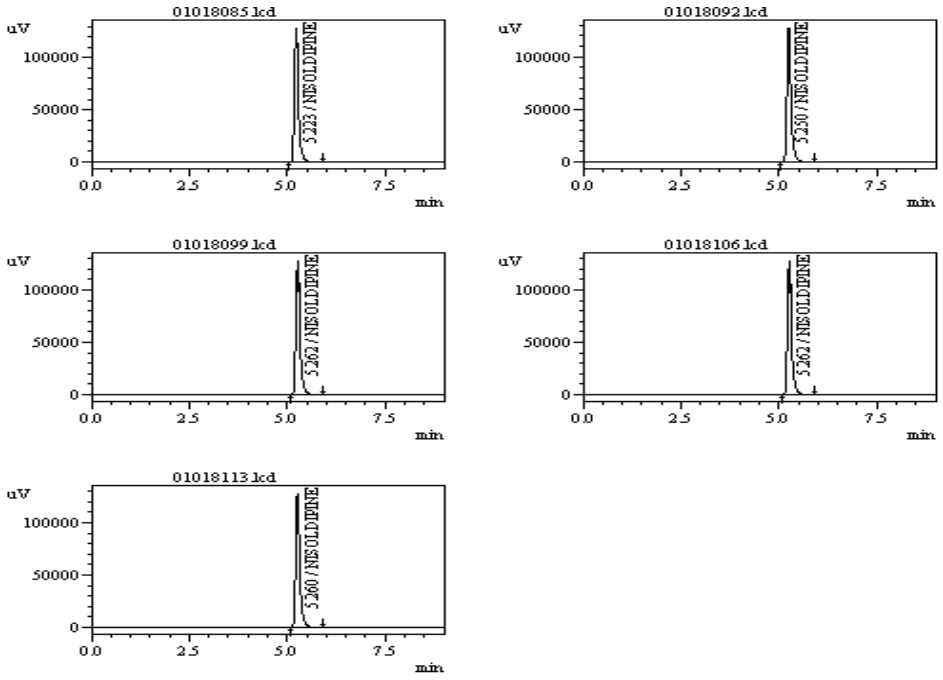


FIG NO 3.1

SPECIFICITY

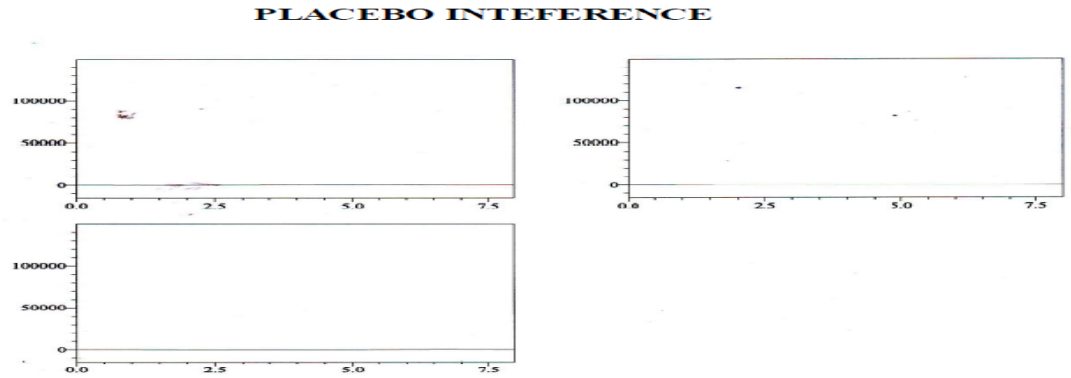
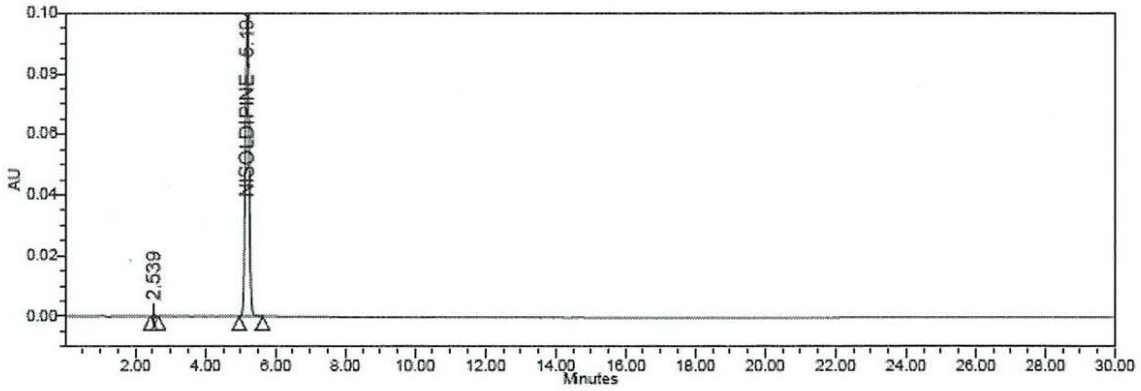


FIG NO: 3.2

Typical chromatogram and Purity Plot of Acid Stressed Drug Substance



Purity Plot

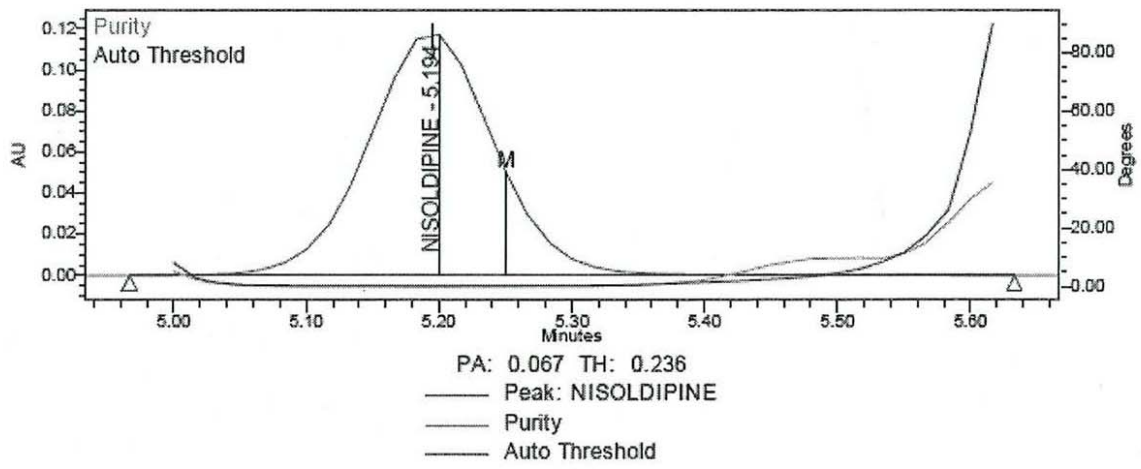
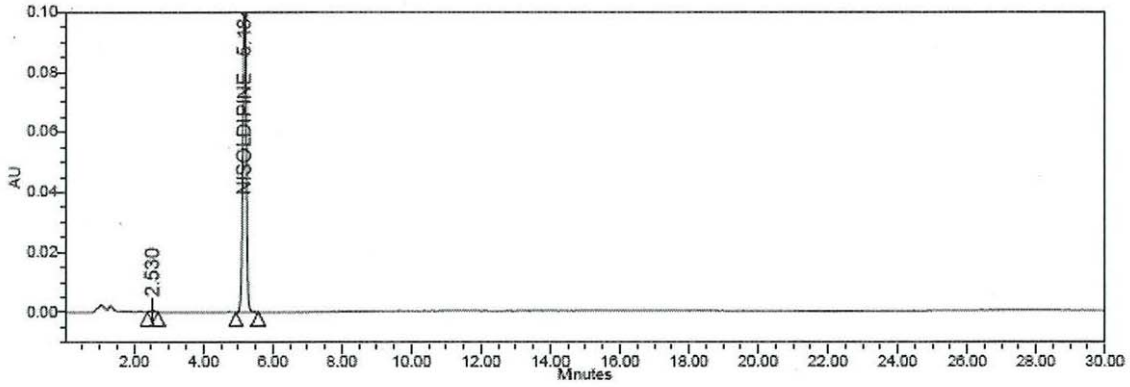


FIG NO: 3.3(A)

Typical chromatogram and Purity Plot of Acid Stressed Drug product



Purity Plot

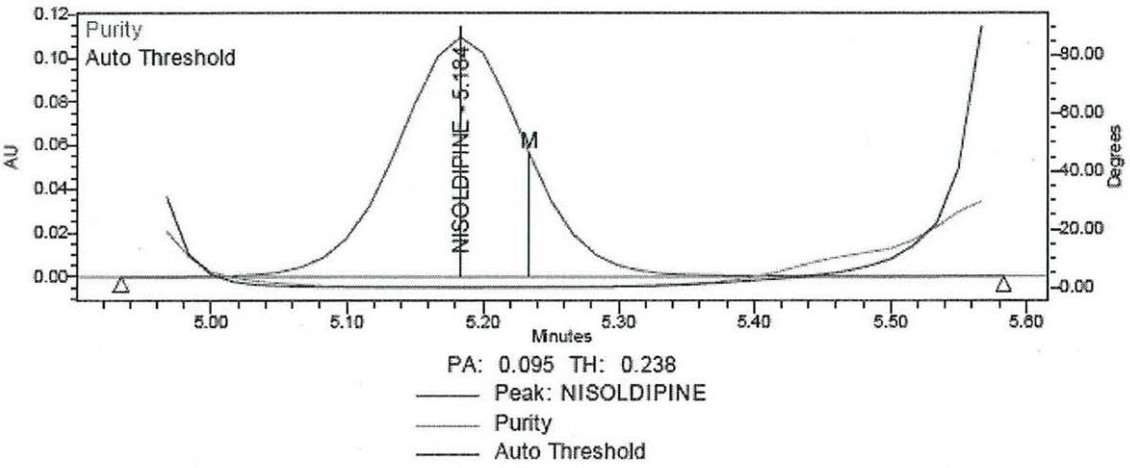


FIG NO: 3.3(B)

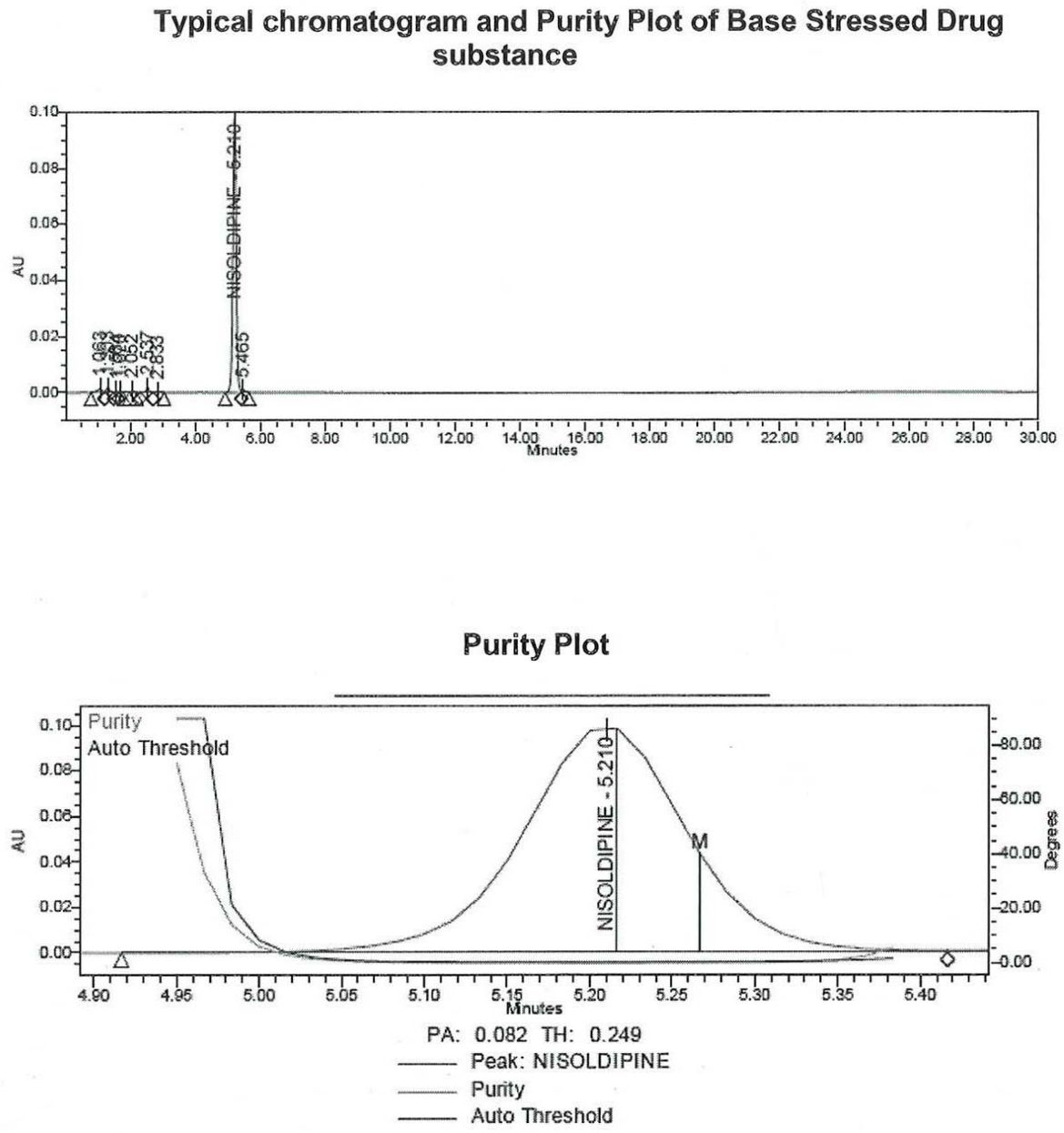

Typical chromatogram and Purity Plot of Base Stressed Drug substance
AU
Minutes
NISOLDIPINE - 5.210
5.465
Purity Plot
Purity
Auto Threshold
NISOLDIPINE - 5.210
M
Degrees
PA: 0.082 TH: 0.249
Peak: NISOLDIPINE
Purity
Auto Threshold

FIG NO: 3.3(C)

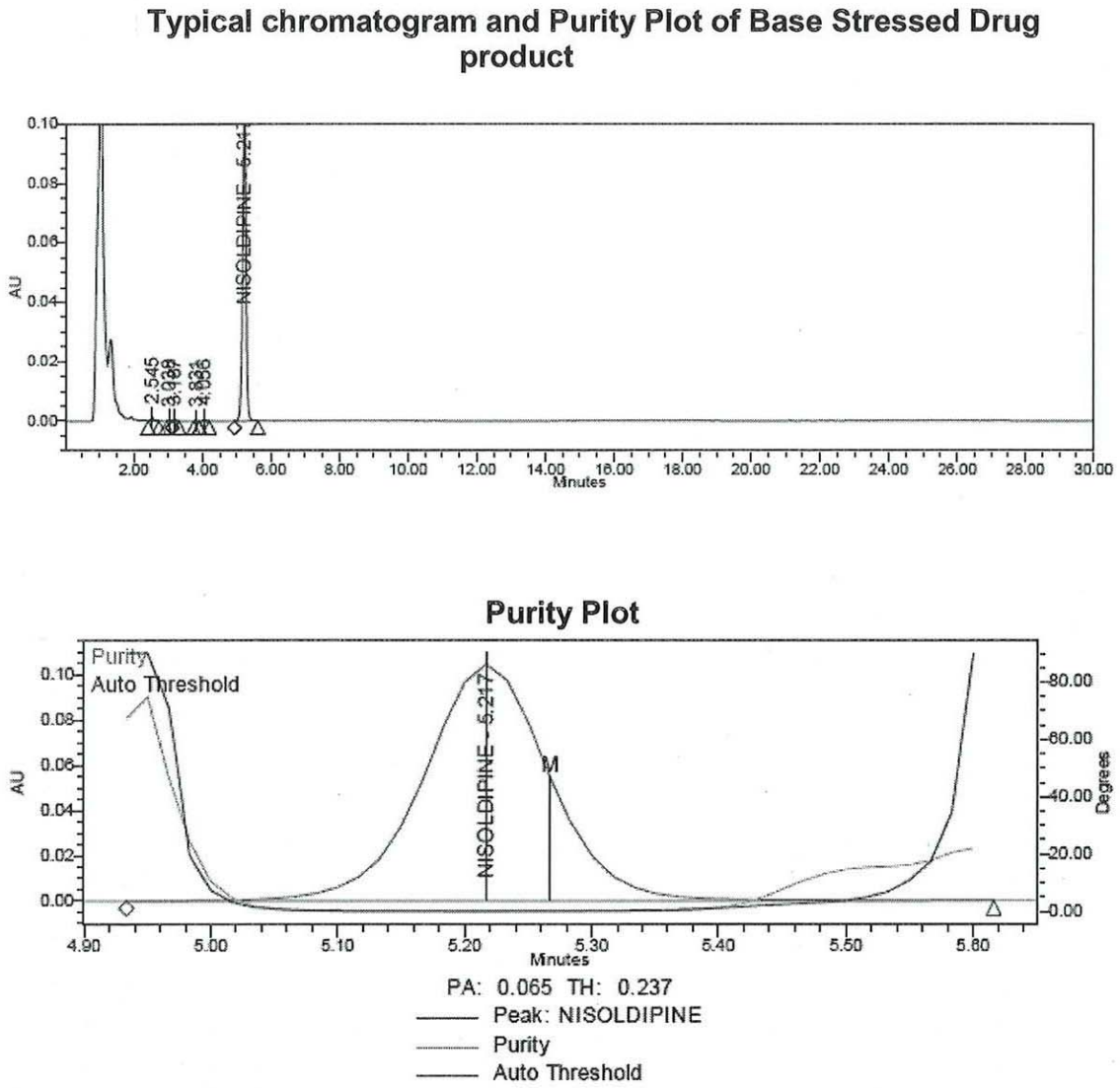
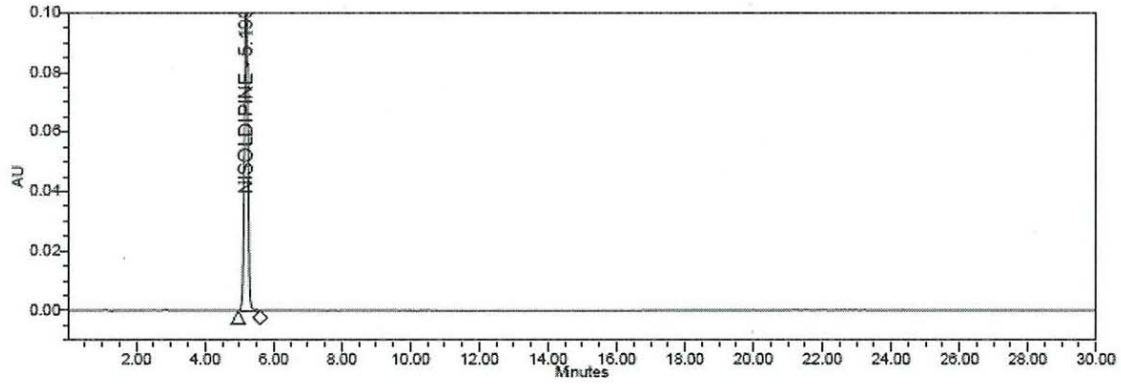

Typical chromatogram and Purity Plot of Base Stressed Drug product
AU
Minutes
NISOLDIPINE - 5.217
Purity Plot
Purity
Auto Threshold
Degrees
PA: 0.065 TH: 0.237
Peak: NISOLDIPINE
Purity
Auto Threshold

FIG NO: 3.3(D)

Typical chromatogram and Purity Plot of Oxidation Stressed Drug Substance



Purity Plot

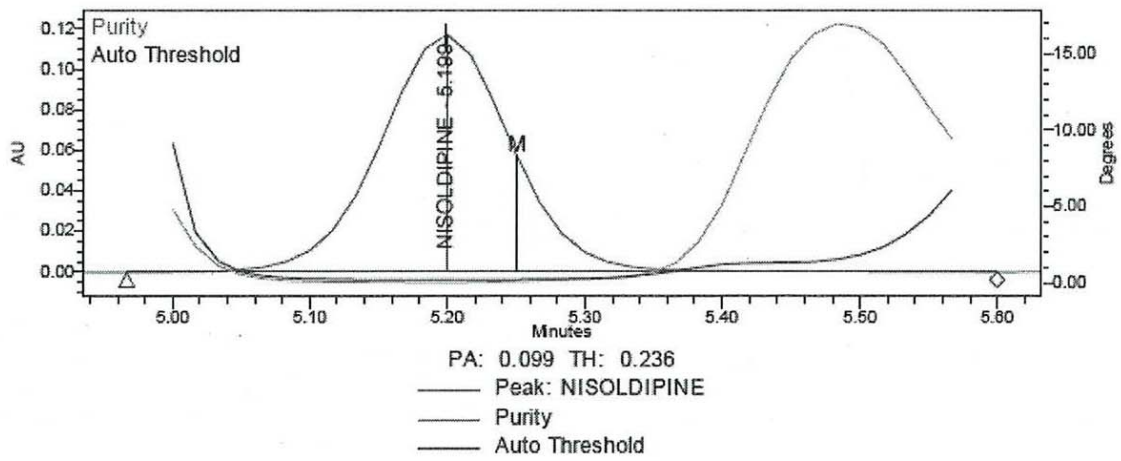


FIG NO: 3.3(E)

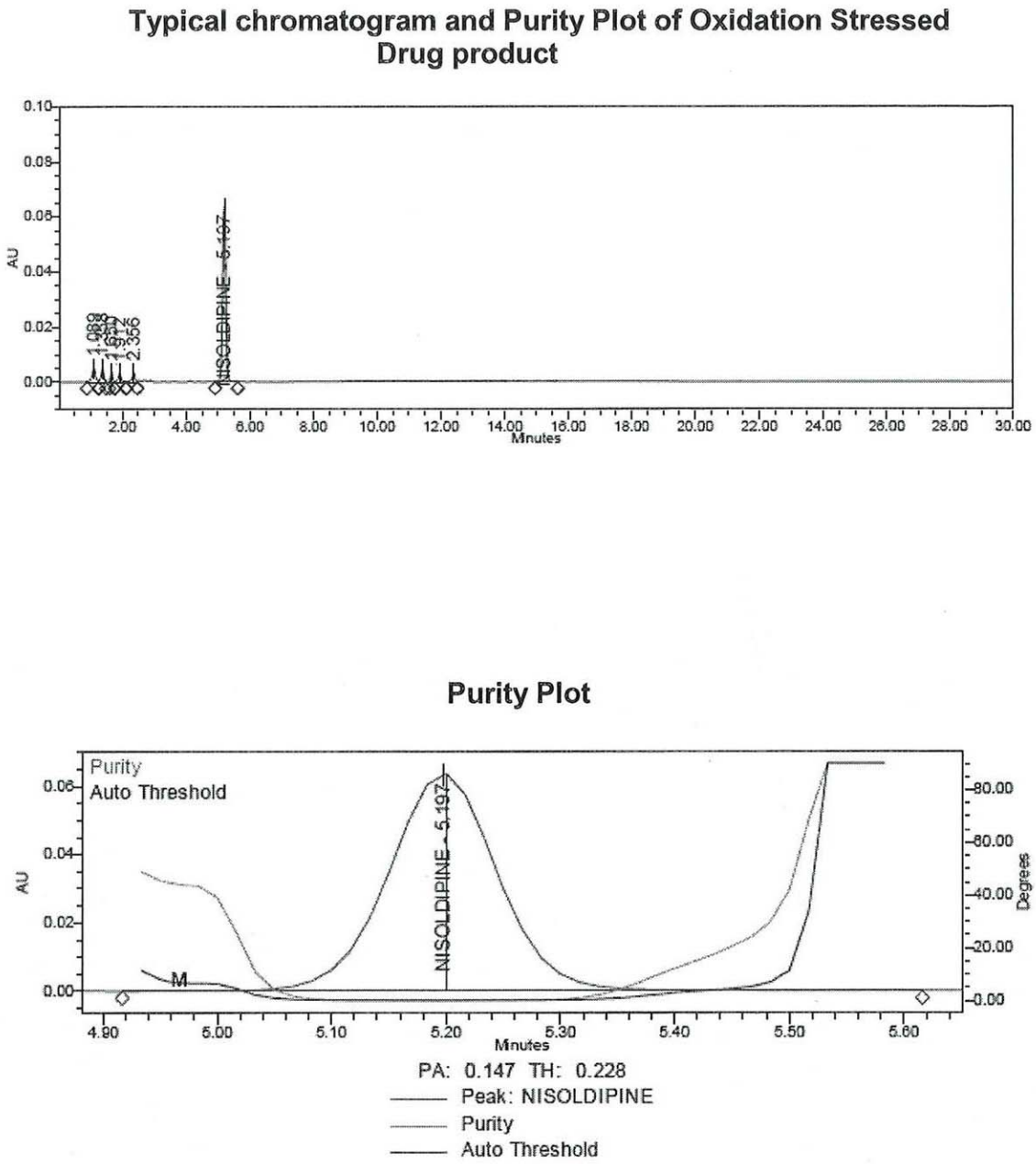
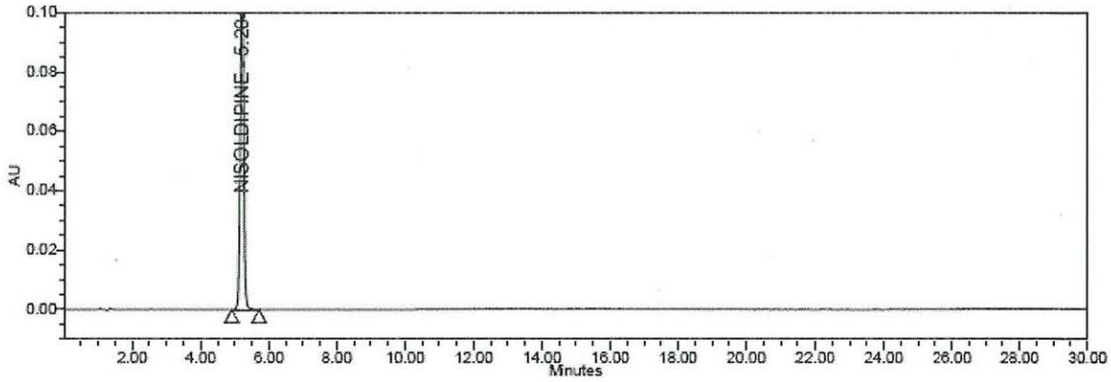

Typical chromatogram and Purity Plot of Oxidation Stressed Drug product
AU
Minutes
NISOLDIPINE - 5.197
Purity Plot
Purity
Auto Threshold
Degrees
M
PA: 0.147 TH: 0.228
Peak: NISOLDIPINE
Purity
Auto Threshold

FIG NO: 3.3(F)

Typical chromatogram and Purity Plot of water Stressed Drug Substance



Purity Plot

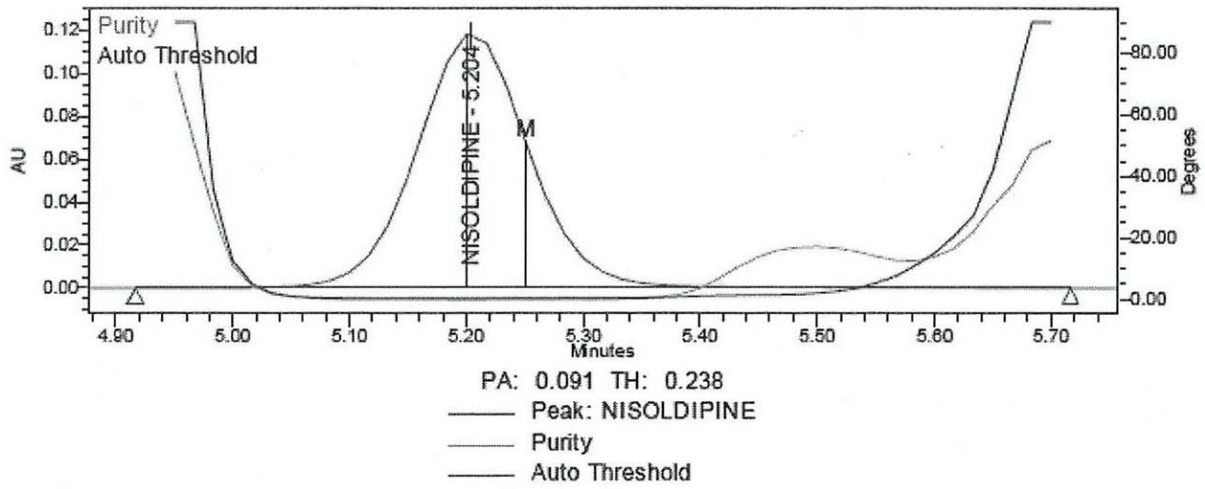
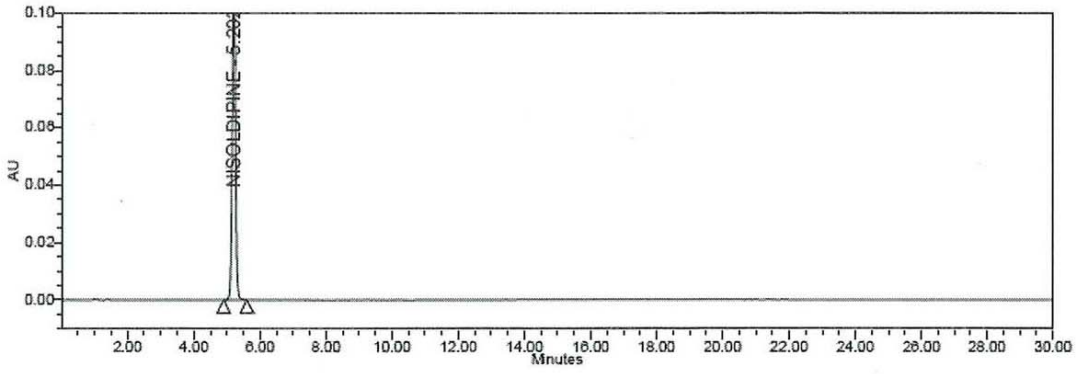


FIG NO: 3.3(G)

Typical chromatogram and Purity Plot of Water Stressed drug product



Purity Plot

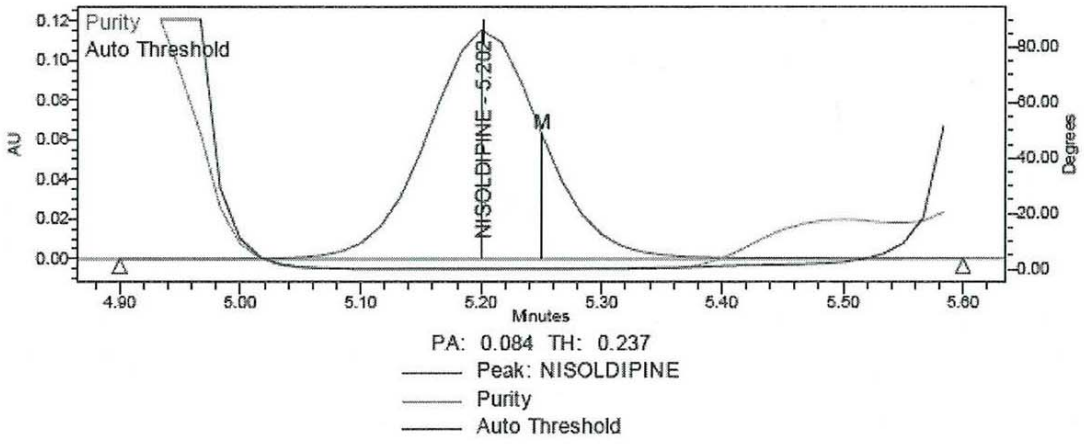
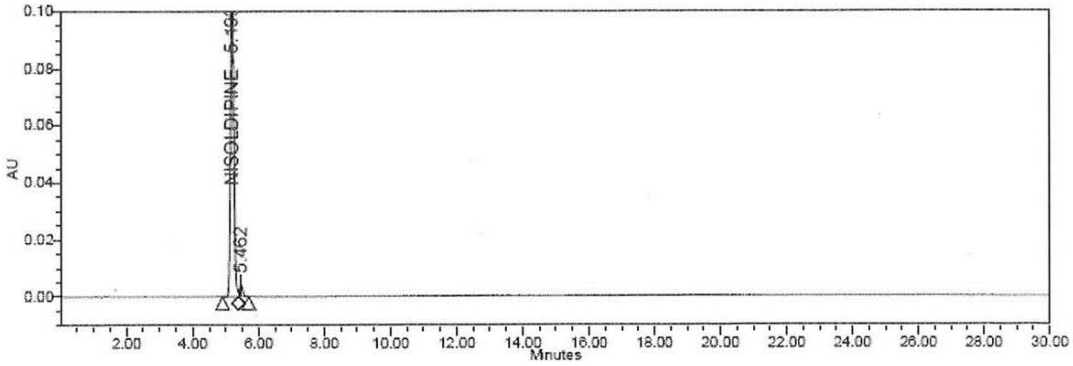


FIG NO: 3.3(H)

Typical chromatogram and Purity Plot of UV Light Stressed Drug Substance



Purity Plot

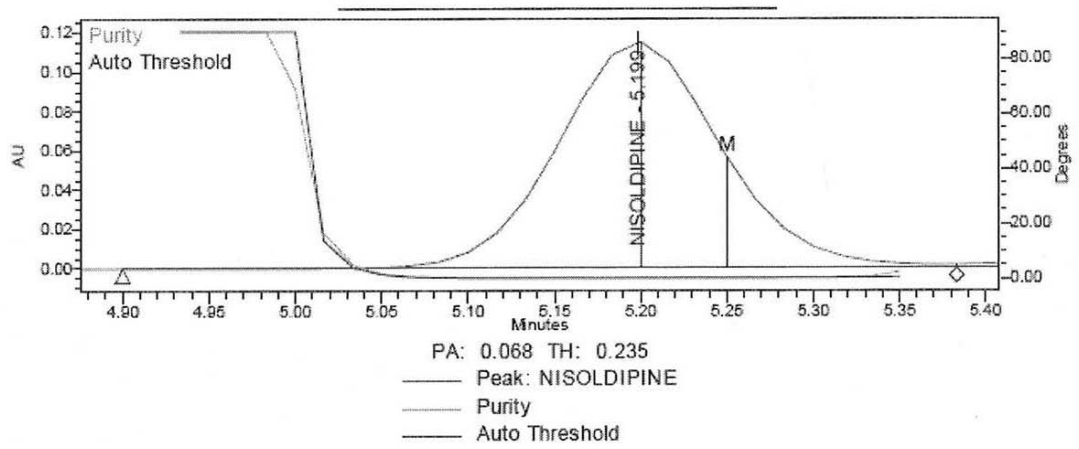
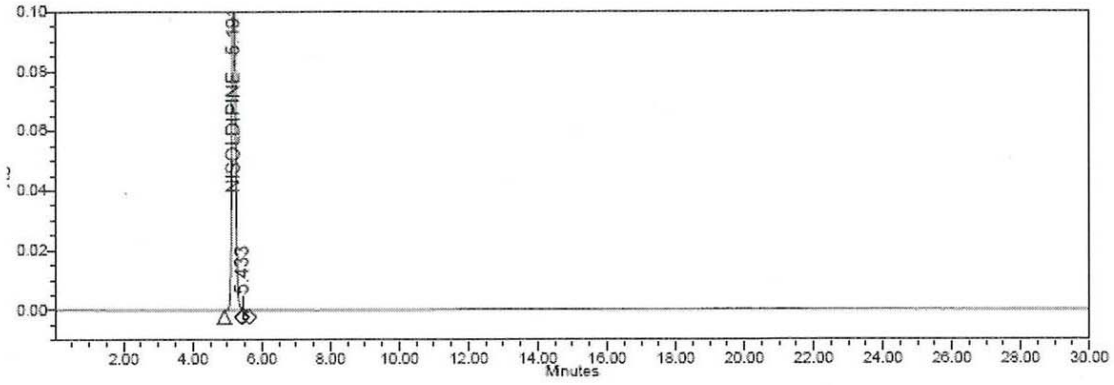


FIG NO: 3.3(I)

Typical chromatogram and Purity Plot of UV Light Stressed Drug product



Purity Plot

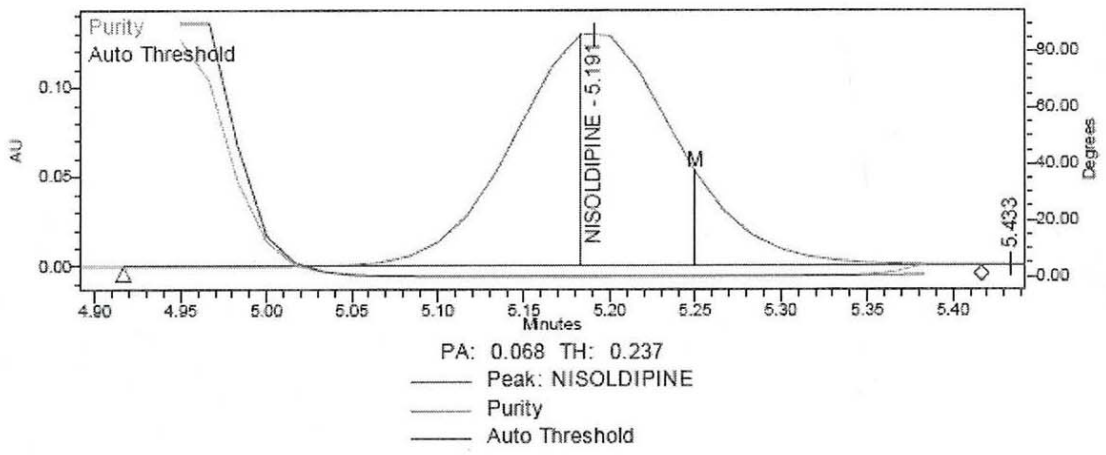
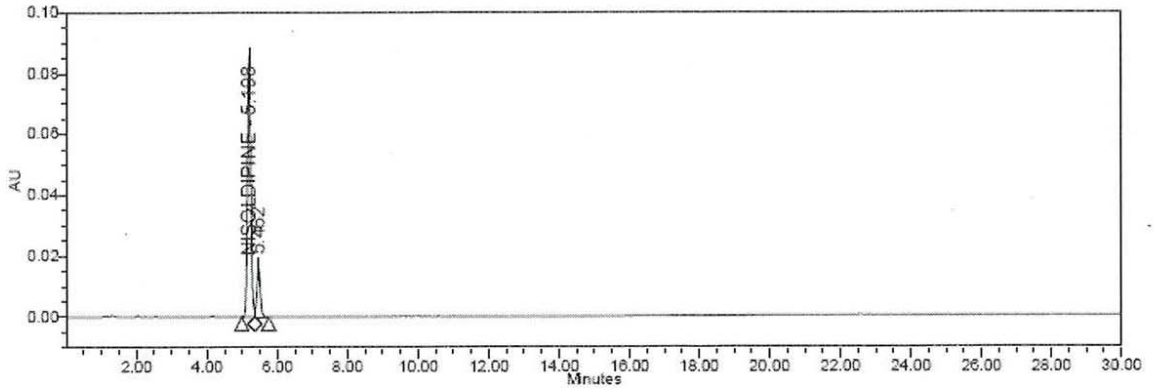


FIG NO: 3.3(J)

Typical chromatogram and Purity Plot of Visible Light Stressed Drug Substance



Purity Plot

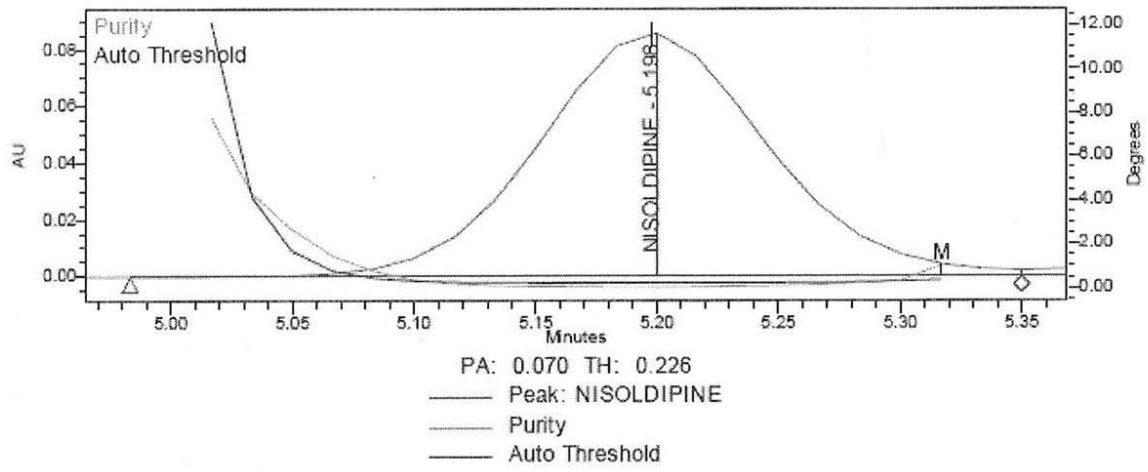
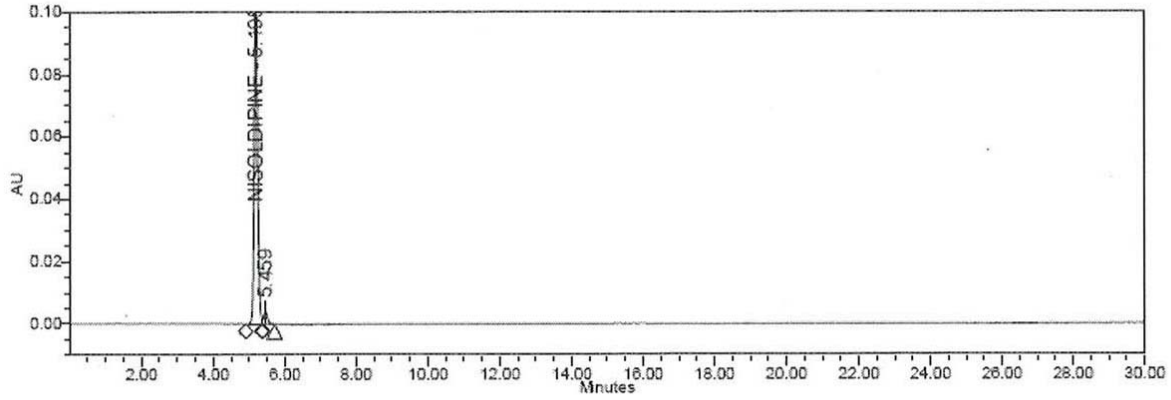


FIG NO: 3.3(K)

Typical chromatogram and Purity Plot of Visible Light Stressed drug product



Purity Plot

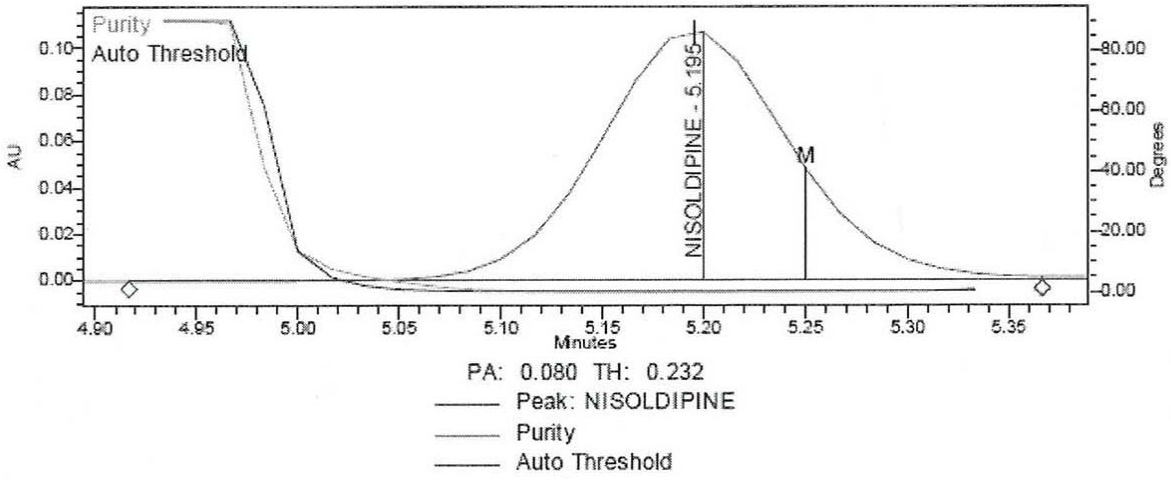


FIG NO: 3.3(L)

METHOD LINEARITY

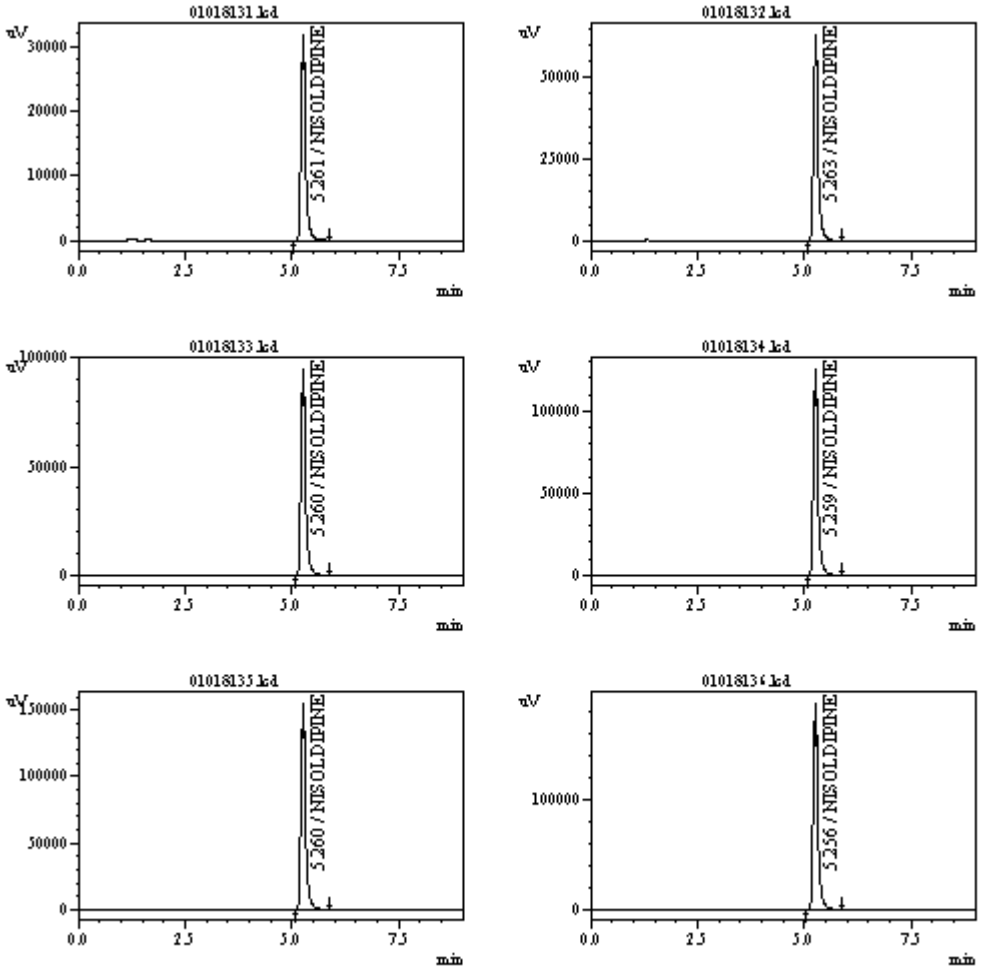


FIG NO: 4

PRECISION

REPEATABILITY

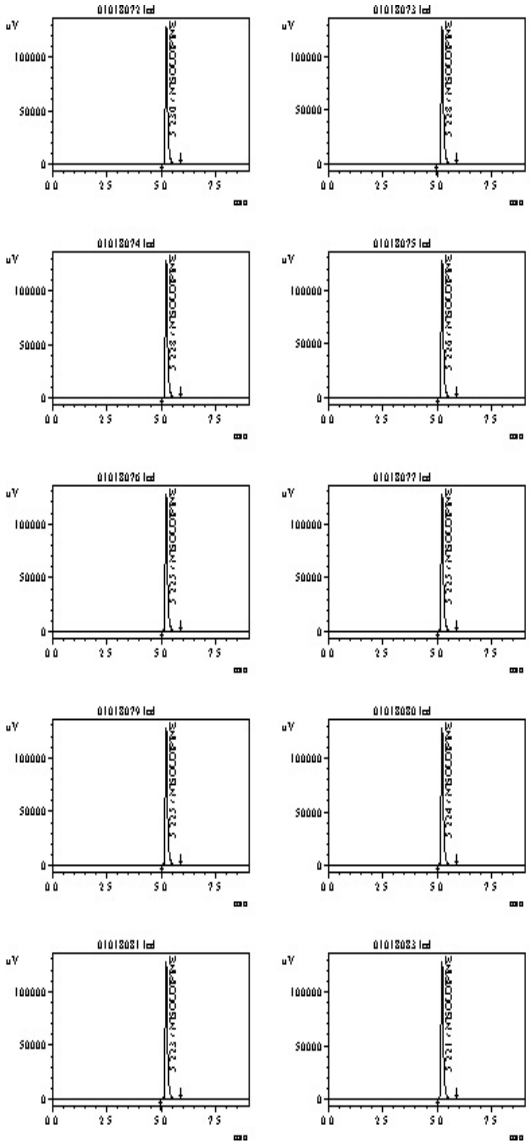


FIG NO: 5

ACCURACY

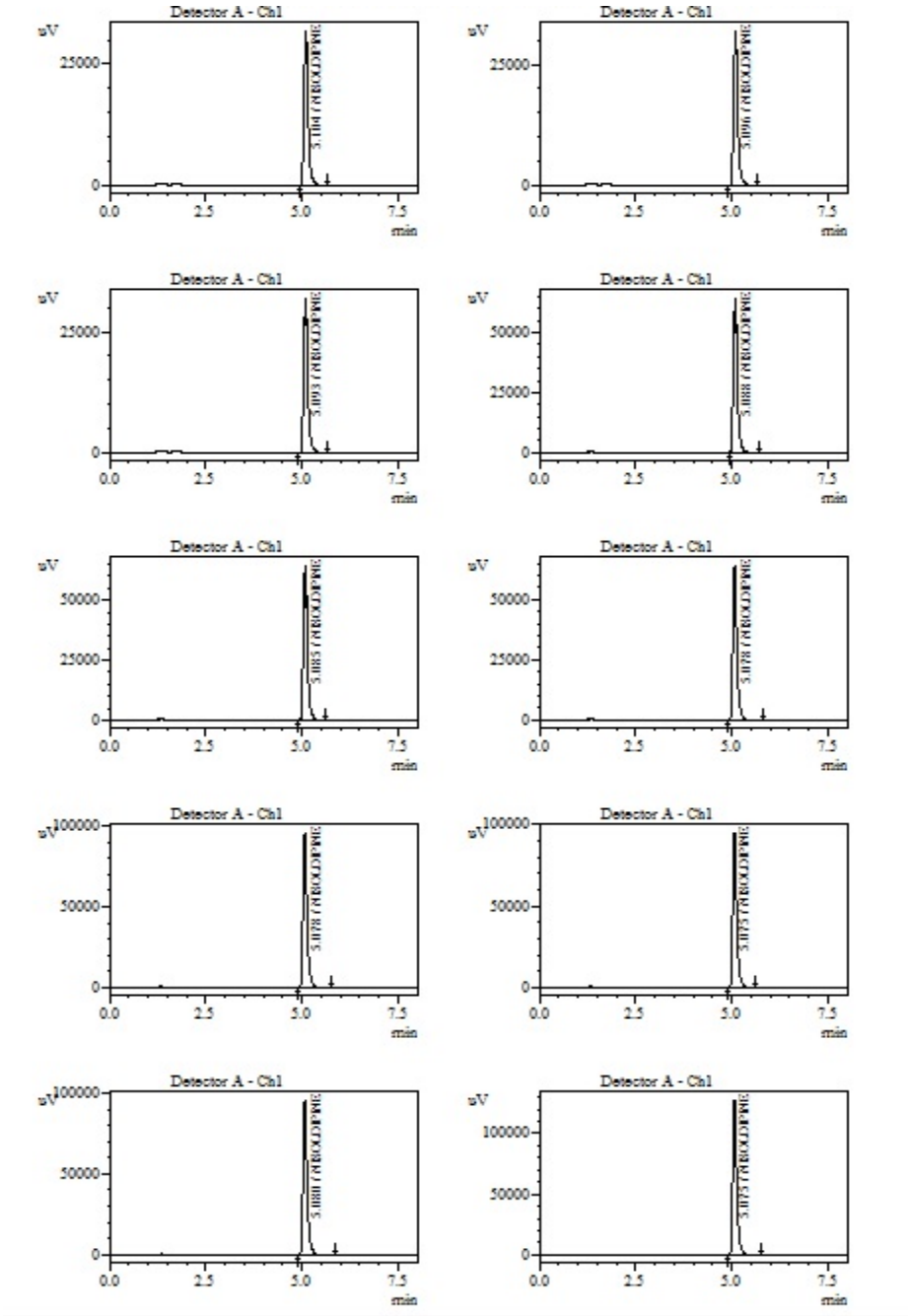


FIG NO: 6

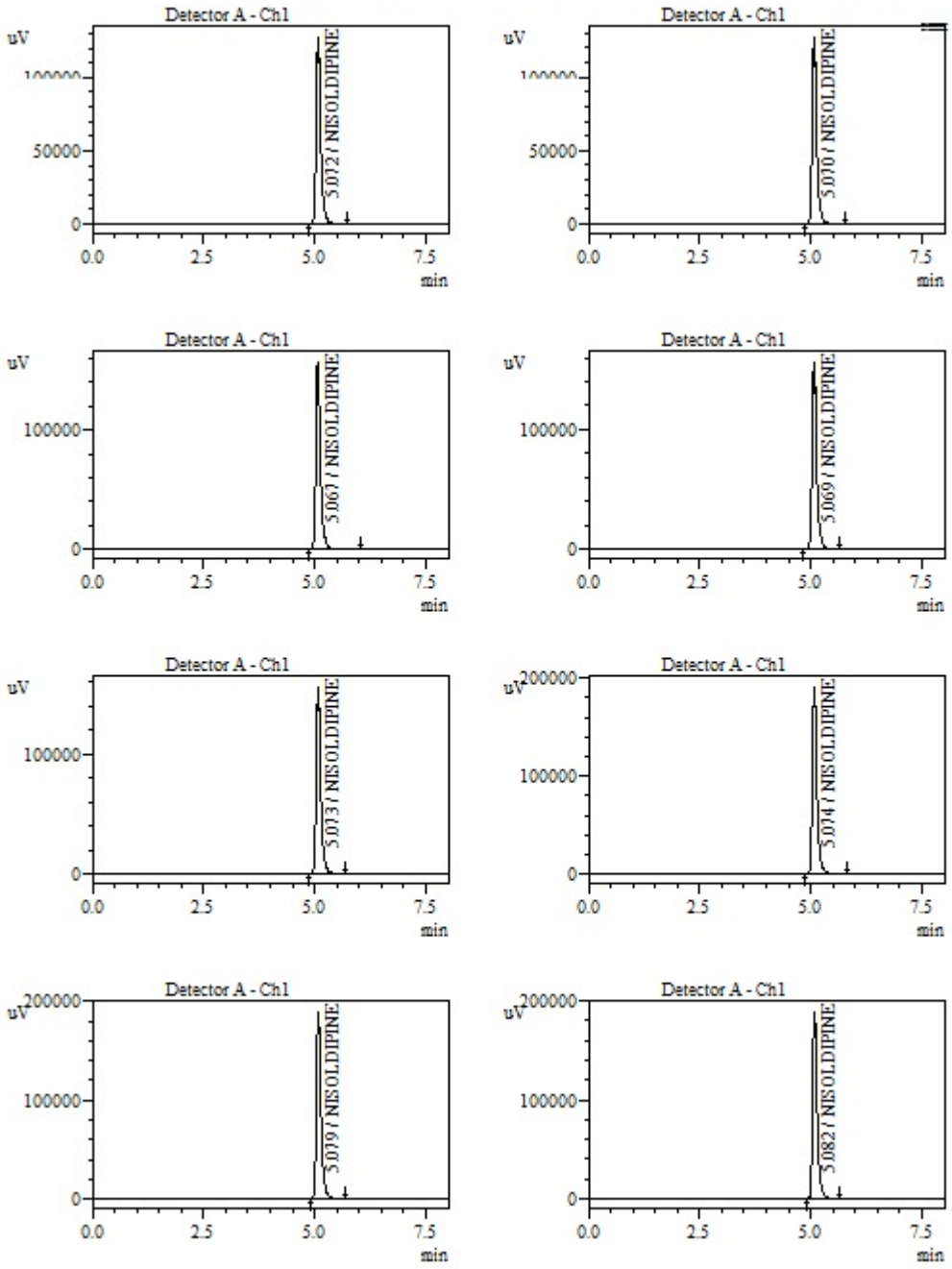
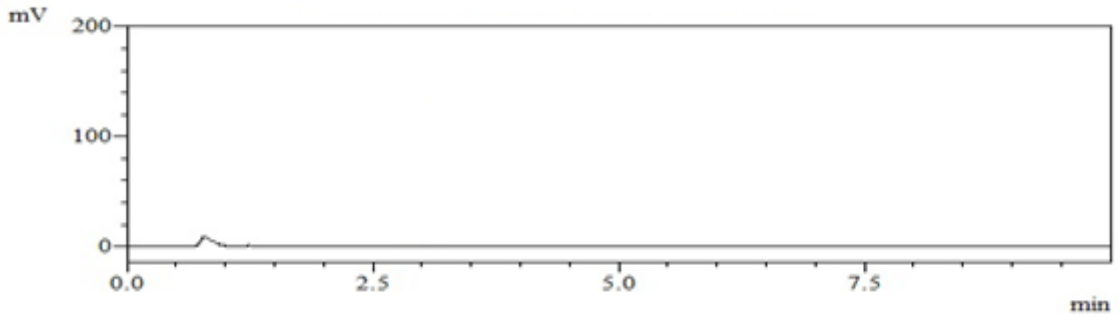
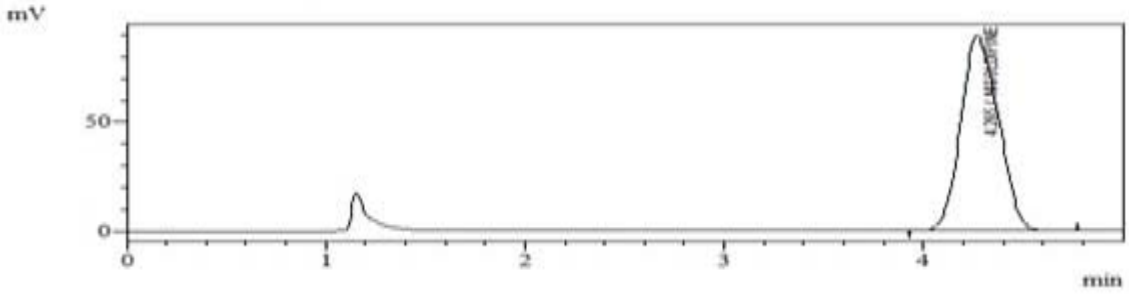

Detector A - Ch1
uV
50000
0
0.0
2.5
5.0
7.5
min
5.072 / NISOLDIPINE
Detector A - Ch1
uV
50000
0
0.0
2.5
5.0
7.5
min
5.070 / NISOLDIPINE
Detector A - Ch1
uV
100000
0
0.0
2.5
5.0
7.5
min
5.067 / NISOLDIPINE
Detector A - Ch1
uV
100000
0
0.0
2.5
5.0
7.5
min
5.069 / NISOLDIPINE
Detector A - Ch1
uV
100000
0
0.0
2.5
5.0
7.5
min
5.073 / NISOLDIPINE
Detector A - Ch1
uV
200000
100000
0
0.0
2.5
5.0
7.5
min
5.074 / NISOLDIPINE
Detector A - Ch1
uV
200000
100000
0
0.0
2.5
5.0
7.5
min
5.079 / NISOLDIPINE
Detector A - Ch1
uV
200000
100000
0
0.0
2.5
5.0
7.5
min
5.082 / NISOLDIPINE

FIG NO: 6

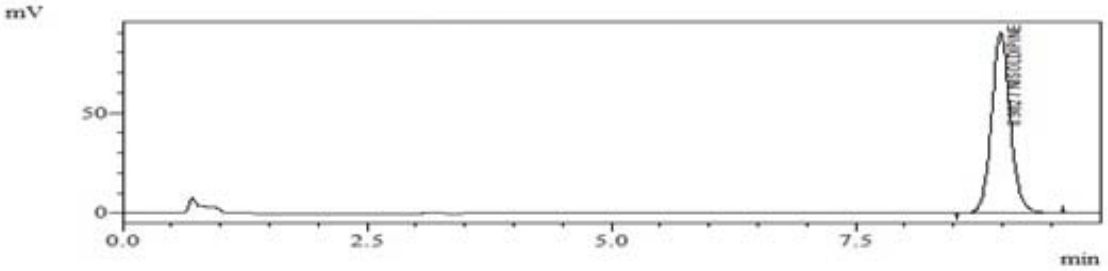
DISSOLUTION



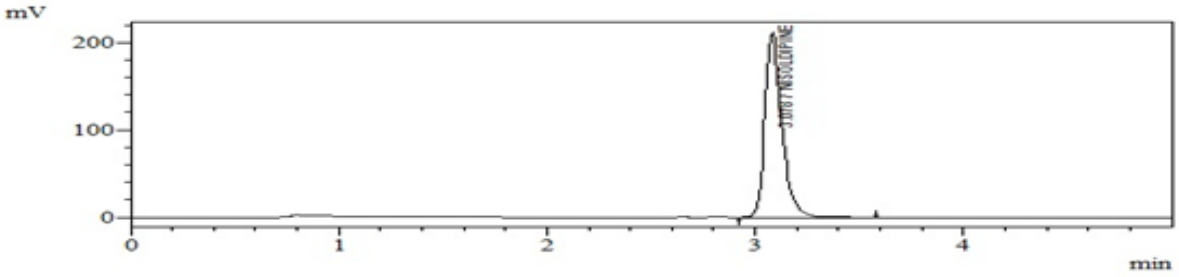
TRAIL-1 (FIG NO: 7.1)



TRAIL-1 (FIG NO: 7.2)



TRAIL-1 (FIG NO: 7.3)



TRAIL-1 (FIG NO: 7.4)

VALIDATION CHROMATOGRAMS-DISSOLOUTION

SYSTEM SUITABILITY

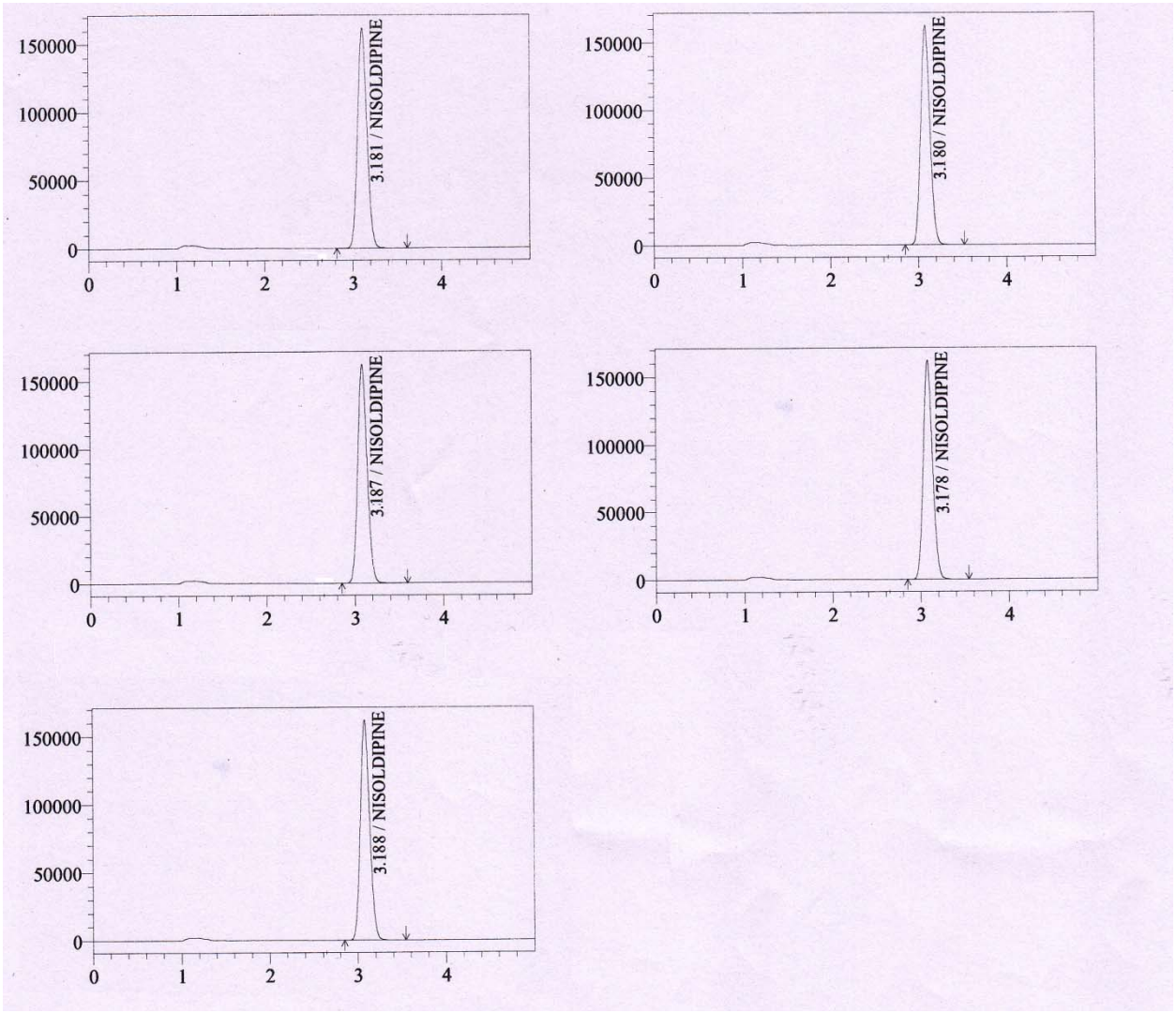


FIG NO: 8

SPECIFICITY

PLACEBO INTERFERENCE

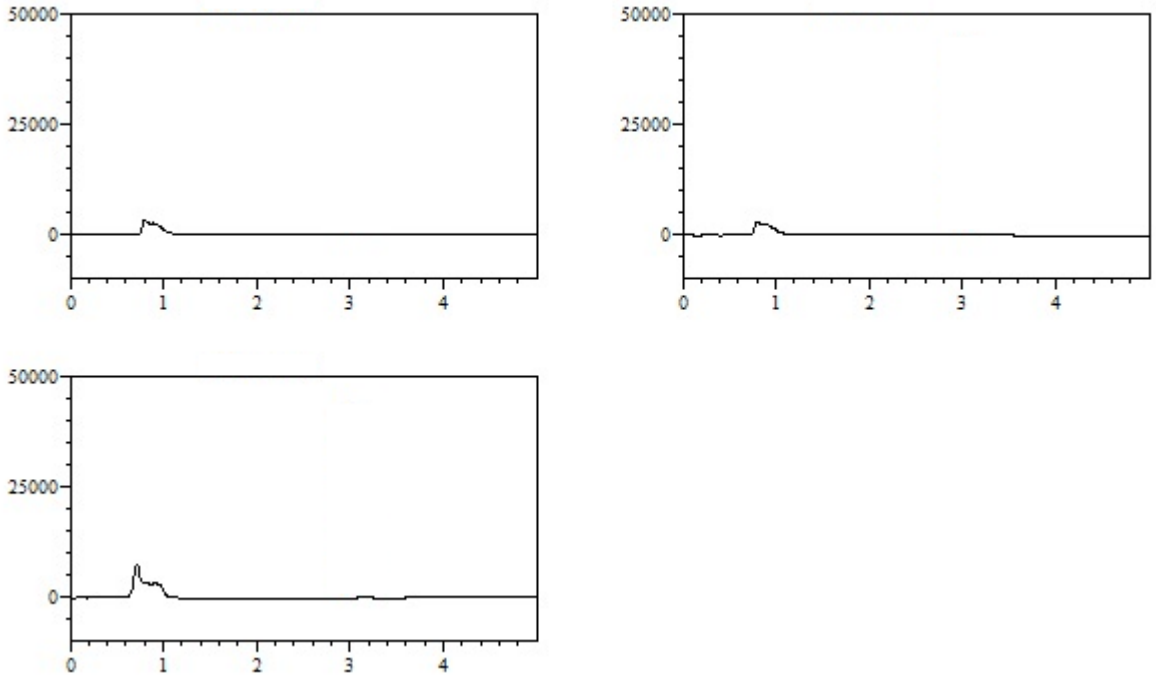


FIG NO: 9

METHOD LINEARITY

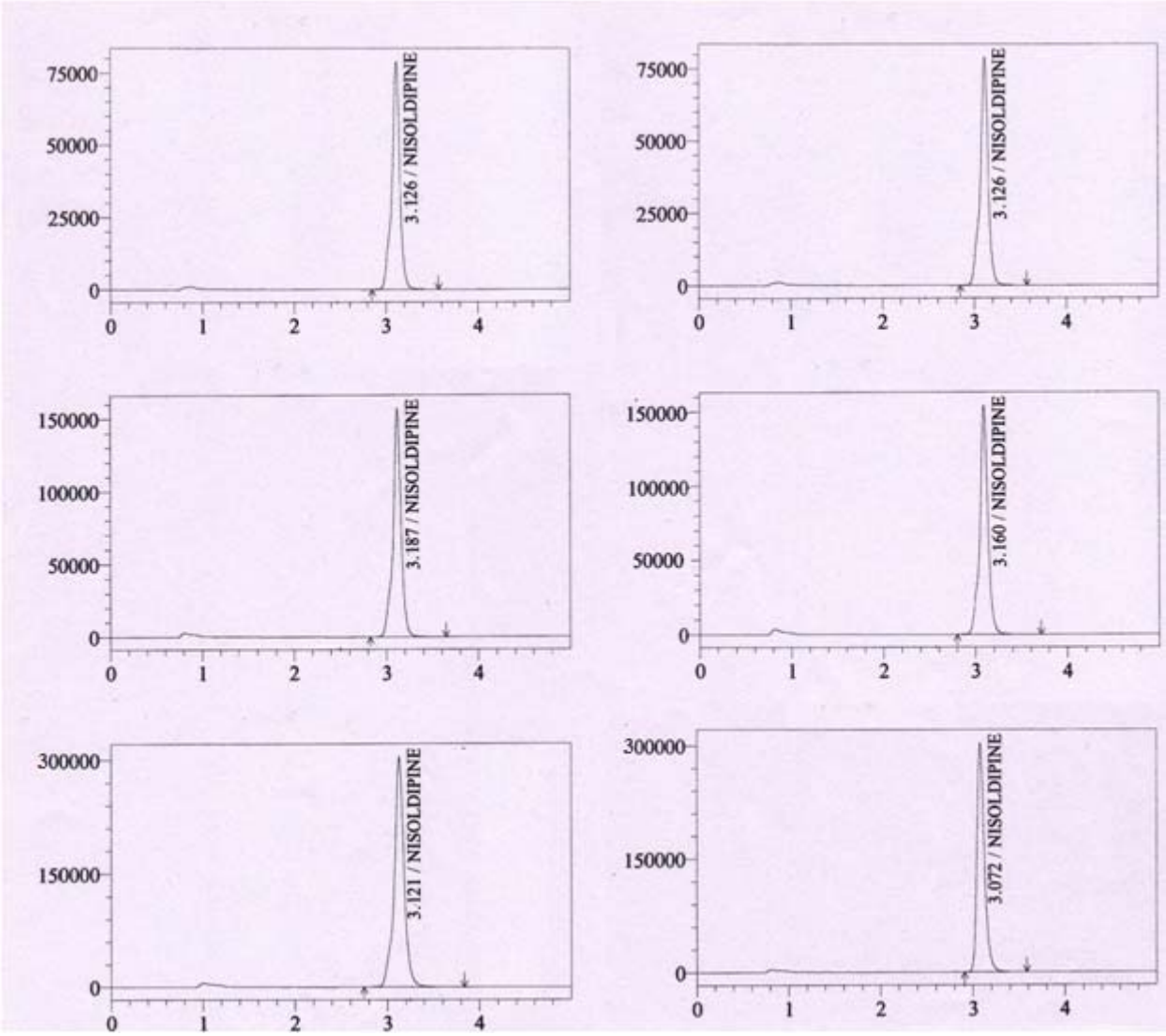


FIG NO: 10

PRECISION

REPEATABILITY

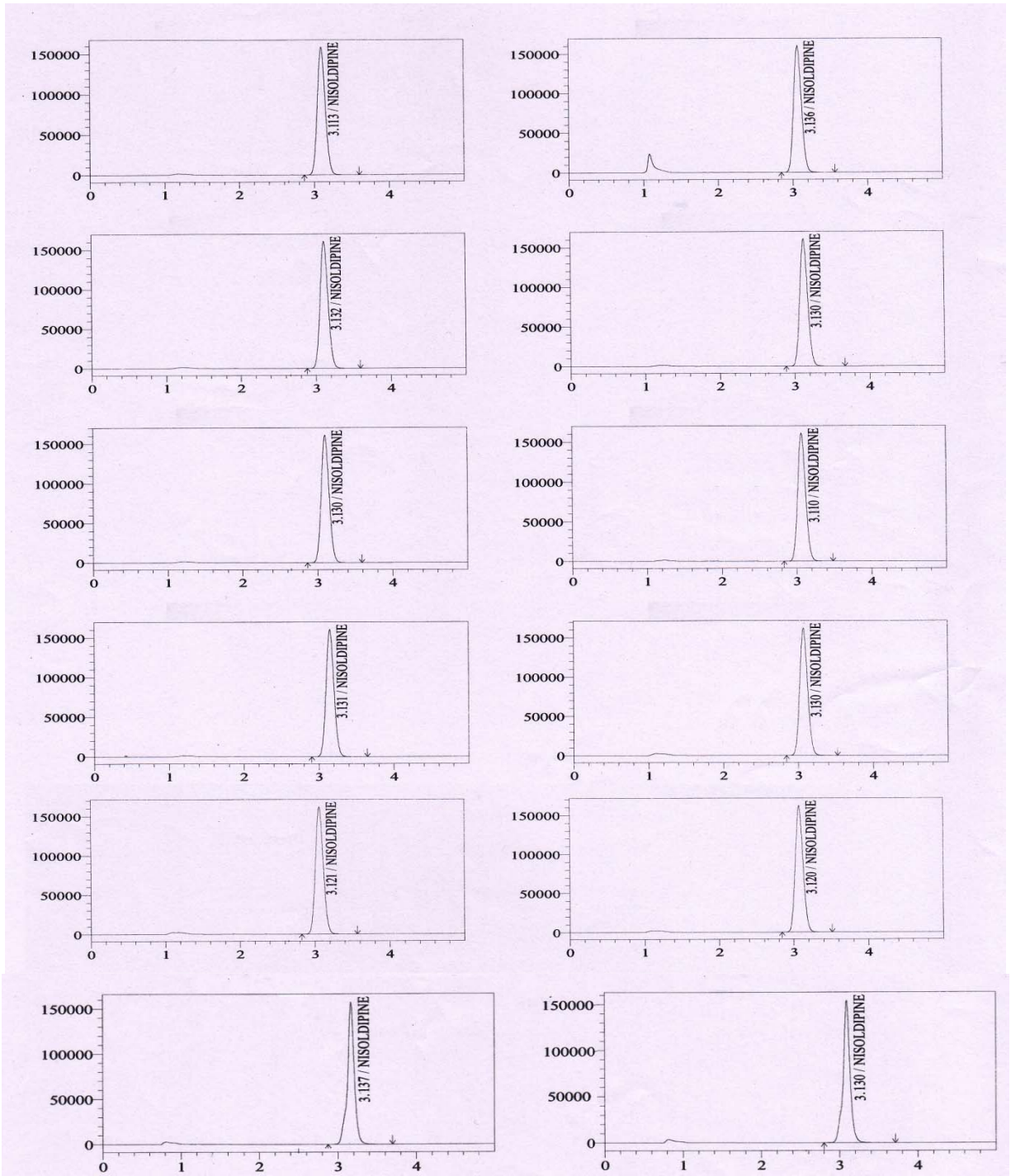


FIG NO: 11

SINK CONDITION

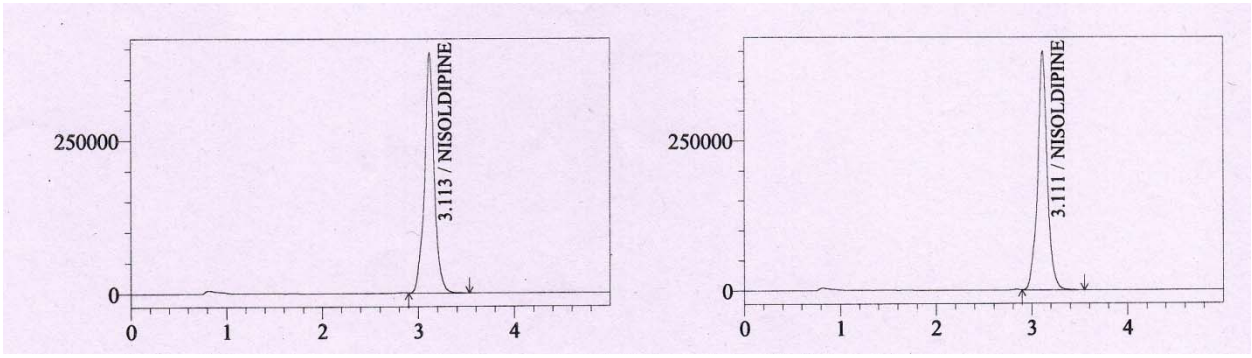


FIG NO: 12

8. SUMMARY AND CONCLUSION

SUMMARY

DEVELOPED CHROMATOGRAPHIC CONDITIONS FOR ASSAY ARE AS FOLLOWS

1. SELECTION OF WAVELENGTH

After reviewing chromatograms and peak purity chromatograms a wavelength of 333nm is selected as the wavelength for this drug. At this wavelength baseline noise is less and peak purity of drug is more.

2. SELECTION OF COLUMN

It was found that the peak shape, retention time, tailing factor, column efficiency are good with Peerless Basic, and hence Peerless Basic C_{18} (100 × 4.6 mm) 1.8µm column is selected. (Table No: 6.1.2)

3. OPTIMIZATION OF MOBILE PHASE COMPOSITION

Good Peak shape, retention time, tailing factor, theoretical plates are obtained with pH 6.8 phosphate buffer: ACN: MeOH (30:40:30) .Hence it was finalized.

4. SELECTION OF FLOW RATE

After reviewing the results it was found that 1.0 mL is resulted with good peak shape, retention time, and good peak symmetries. Hence it was taken as the flow rate for the method. (Table No: 6.1.3)

5. SELECTION OF COLUMN TEMPERATURE

Retention time, peak area, plate count was found to be good at 30°c.(Table No:6.1.4)

6. OPTIMIZATION OF SAMPLE PREPARATION

From this data it is concluded that preparation of test is to be done MeOH: pH3 buffer (50:50). The % assay is more when the sample preparation was done in MeOH: pH3 buffer (50:50), hence this composition was selected as diluent.

7. ADJUSTING THE INJECTION VOLUME

Good peak shape, retention time, and more theoretical plates are obtained when an injection volume of 20 µl was used.(Table No: 6.1.5)

8. ESTABLISHMENT OF SYSTEM SUITABILITY

As the % RSD is below 2% .we can say the chromatographic system is adequate for the analysis to be done.

9. INSTRUMENT

Shimadzu HPLC system with UV detector and auto sampler.

VALIDATION OF ASSAY PARAMETERS

1. SYSTEM PRECISION

The %RSD of ten injections was found to be 0.0% (acceptance criteria 1.0%), it concludes the system precision was passed. (Table No 6.2.1, Fig No 3)

System Suitability (Table No 6.2.2, Fig No 3.1)

All the system suitability parameters are passed

2. SPECIFICITY

a. Placebo Interference

Main peak have no interference with placebo peak, hence no placebo interference. (Table No 6.2.3, Fig No 3.2)

b. Interference From Degradants

Purity angle is less than purity threshold and Nisoldipine peak does not have any flag in purity results. (Table No 6.2.4, Fig No 3.3(A) - Fig No 3.3(L)

3. LINEARITY OF METHOD

The coefficient of correlation was found to be 0.9998, hence the method response was found to be linear. (Table No 6.2.5, Fig No 4, Chart No: 1)

4. PRECISION

Repeatability

The relative standard deviation of % assay results was found to be 0.10, and the % assay results of Nisoldipine are within 95% and 105%, hence it was concluded that the method shows repeatability. (Table No 6.2.6, Fig No 5)

5. ACCURACY

The average % recovery of Nisoldipine at each spike level was not less than 95% and not more than 105%, hence it was concluded that the method shows accuracy. (Table No 6.2.7, Fig No 6)

6. **RUGGEDNESS**

a. Analyst To Analyst Variability

b. All the individual assay values of Nisoldipine are within 95% to 105% and the relative standard deviation of % assay results are not more than 2%, hence there is no analyst to analyst variability. (Table No 6.2.8&6.2.9)

c. System To System Variability

All the individual assay values of Nisoldipine are within 95% to 105% and the relative standard deviation of % assay results are not more than 2%, hence there is no system to system variability. (Table No 6.2.10&6.2.11)

d. Column to column variability

All the individual assay values of Nisoldipine are within 95% to 105% and the relative standard deviation of % assay results are not more than 2%, hence there is no column to column variability. (Table No 6.2.12&6.2.13)

e. Bench top stability of mobile phase

The % assay results of Nisoldipine not differ from initial value by more than 2%, hence it was concluded the mobile phase was stable for two days. (Table No 6.2.14&6.2.15)

f. Bench top stability of Nisoldipine standard and test solution

The % assay of Nisoldipine in standard and test preparation does not differ by more than 2% from initial value, hence it was concluded the standard and the sample solutions were stable for two days. (Table No 6.2.16)

g. Refrigerator stability of samples

The % assay of Nisoldipine in standard and test preparation does not differ by more than 2% from initial value, hence it was concluded that the standard and sample solutions were stable for two days under refrigeration. (Table No 6.2.17)

6. ROBUSTNESS

a. Effect of variation in mobile phase composition

The average % assay values with the variation of 10% composition of organic phase does not differ by more than 2%when compared to test method values ,hence it was

concluded that there is no effect of variation in mobile phase composition on % assay. (Table No 6.2.18&6.2.19)

b. Effect of variation in flow rate

The average % assay values with the variation of ±0.1mL/min does not differ by more than 2% when compared to test method values, hence it was concluded that there is no effect of variation in flow rate on % assay. (Table No 6.2.20&6.2.21)

c. Effect of variation in pH of buffer in mobile phase

The average % assay values with the variation of ±0.2 pH does not differ by more than 2% when compared to test method values, hence it was concluded that there is no effect of variation in pH on % assay. (Table No 6.2.22&6.2.23)

d. Effect of variation in column temperature

The average % assay values with the variation of 5°c of column temperature does not differ by more than 2% when compared to test method values, hence it was concluded that there is no effect of variation in column temperature on % assay. (Table No 6.2.24&6.2.25)

7. FILTER VALIDATION

The difference in % assay of filtered sample and centrifuged sample is not more than 2 %, hence it was concluded that there is no interference due to filters. (Table No 6.2.26&6.2.27)

Results of Assay Method Validation of Nisoldipine Tablets

Table No: 8.1.1

S.No	Parameters	Observation	Acceptance Criteria	Passes/ Fail
1. 1.1 1.2	Precision System Precision Method Precision	 0.15 0.10	Percentage relative standard deviation (%RSD) is not more than 2.0	Passes
2.	Specificity	No peak elute	No peak elutes at the retention time of main peak in the blank and placebo	Passes
3.	Forced Degradation a. Degradation By Hydrochloric Acid b. Degradation By Sodium Hydroxide c. Degradation By Hydrogen Per-Oxide d. Degradation by Thermal Heat. e. Degradation By Exposing Light	Well within the limit	Purity angle should be less than purity threshold.	Passes
4.	Linearity	Correlation coefficient-0.9998	Correlation coefficient (r) is not less than 0.999	Passes
5.	Accuracy	The percentage recovery was found between 99.6 – 99.9%	The percentage Recovery at each level is between 98.0-102.0%	Passes
6.	Solution Stability	Well within the limit	Cumulative %RSD is not more than 2.0	Passes
7.	Filter Interference	Well within the limit	The percentage difference is not more than 2.0	Passes
8.	Robustness	Well within the limit	Overall percentage Relative standard deviation (%RSD) is not more than 2.0	Passes

DEVELOPED CHROMATOGRAPHIC CONDITIONS FOR DISSOLUTION ARE AS FOLLOWS

1. **SELECTION OF WAVELENGTH**

   After reviewing chromatograms and peak purity chromatograms a wavelength of 333nm is selected as the wavelength for this drug. At this wavelength the response of the drug is more and no interferences.

2. **SELECTION OF COLUMN**

   It was found that the peak shape, retention time, tailing factor, column efficiency are good with Prontosil H, and hence Prontosil (100 × 4.6 mm) 3µm column is selected. (Table No: 6.3.2)

3. **OPTIMIZATION OF MOBILE PHASE COMPOSITION**

   Good Peak shape, retention time, tailing factor, theoretical plates are obtained with pH 3buffer: ACN: MeOH (30:40:30) .Hence it was finalized.

4. **SELECTION OF FLOW RATE**

   After reviewing the results it was found that 1.5 mL is resulted with good peak shape, retention time, and good peak symmetries. Hence it was finalized. (Table No: 6.3.3)

5. **SELECTION OF COLUMN TEMPERATURE**

   Retention time, peak area, plate count was found to be good at ambient temperature.(Table No: 6.3.4)

6. **OPTIMIZATION OF SAMPLE PREPARATION**

   As the % drug release is more in 0.1 N HCL+0.5%SLS, it was selected as diluent for sample preparation.

7. **ADJUSTING THE INJECTION VOLUME**

   Good peak shape, retention time, and more theoretical plates are obtained when an injection volume of 50 µl was used.

8. **OPTIMIZATION OF STANDARD PREPARATION**

   From the solubility studies maximum solubility of drug was found in 0.1 N HCL+0.5%SLS, hence it was used as diluent for extraction of drug.

8. SELECTION OF DISSOLUTION PARAMETERS

Dissolution parameters like Medium, RPM, Medium volume, Time points, Apparatus can select from office of generic drug and confirmed by similarity factor studies.(Table No:6.3.7(a)-6.3.7(e)&Chart No:2-6).

VALIDATION OF DISSOLUTION METHOD

1. SYSTEM PRECISION

The %RSD of ten injections was found to be 0.38 (acceptance criteria 1.0%), it concludes the system precision was passed. (Table No 6.4.1)

System Suitability

All the system suitability parameters are passed (Table No 6.4.2, Fig No 8)

2. SPECIFICITY

Placebo Interference

Main peak have no interference with placebo peak, hence no placebo interference. (Table No 6.2.3, Fig No 9)

3. PRECISION OF THE TEST METHOD

Repeatability : The average % dissolution values are not less than 95%,and the relative standard deviation of dissolution values was found to be 0.1%(acceptance limit 5%),it can be concluded that the method shows repeatability. (Table No 6.4.4, Fig No 11)

4. ACCURACY

The average % recovery of Nisoldipine at each spike level was not less than 95%, hence it was concluded that the method shows accuracy. (Table No 6.4.5)

5. LINEARITY OF TEST METHOD

As the correlation coefficient was found to be 0.9997, it was concluded the developed method shows linearity. (Table No 6.4.6, Fig No 10, Chart No: 7)

6. RUGGEDNESS

a. System to System Variability

The average dissolution values are not less than 95%, and the relative standard deviation of dissolution are not more than 2% by both the systems, hence it was concluded that there is no system to system variability. (Table No 6.4.7&6.4.8)

b. Column to column variability

The average dissolution values are not less than 95%, and the relative standard deviation of dissolution are not more than 2% by both the columns, hence it was concluded that there is no column to column variability. (Table No 6.4.9&6.4.10)

c. Analyst to Analyst Variability

The average dissolution values are not less than 95%, and the relative standard deviation of dissolution are not more than 2% by both the analysts, hence it was concluded that there is no analyst to analyst variability. (Table No 6.4.11&6.4.12)

7. ROBUSTNESS

a. Effect of variation in flow rate

The average % assay values with the variation of ±0.1mL/min does not differ by more than 2% when compared to test method values, hence it was concluded that there is no effect of variation in flow rate on % assay. (Table No 6.2.13&6.2.14)

b. Effect of variation in pH of buffer in mobile phase

The average % assay values with the variation of ±0.1 pH does not differ by more than 2% when compared to test method values, hence it was concluded that there is no effect of variation in pH on % assay. (Table No 6.2.15&6.2.16)

c. Filter Validation

The difference between % dissolution of filtered sample and centrifuged sample is not more than 2 %, hence it was concluded that there is no interference due to filters. (Table No 6.4.17&6.4.18)

8. SINK CONDITION

'mg' of Nisoldipine recovered is not less than 60mg. (Table No 6.4.19, Fig No 12)

Results of Dissolution Method Validation of Nisoldipine Tablets

Table No: 8.1.2

S.No	Parameters	Observation	Acceptance Criteria	Passes/ Fail
1. 1.1 1.2	Precision System Precision Method Precision	 0.38 0.50	Percentage relative standard deviation (%RSD) is not more than 2.0	Passes
2.	Specificity	No peak elute	No peak elutes at the retention time of main peak in the blank and placebo	Passes
3.	Linearity	Correlation coefficient is 0.9997	Correlation coefficient (r) is not less than 0.999	Passes
4.	Accuracy	The percentage recovery was found between 99.4 – 100.1%	The percentage Recovery at each level is between 95.0-105.0%	Passes
5.	Filter Interference	Well within the limit	The percentage difference is not more than 2.0	Passes
6.	Robustness	Well within the limit	Overall percentage Relative standard deviation (%RSD) is not more than 2.0	Passes
7.	Sink condition	The amount of Nisoldipine dissolved 80mg.	The amount of Nisoldipine dissolved shall not be less than 60mg.	Passes

CONCLUSIONS

ASSAY

An isocratic **RP- HPLC** method for analysis of Nisoldipine in pharmaceutical dosage form has been developed and validated. Best separation was achieved on a **PEERLESS BASIC C_{18}** (100 × 4.6 mm) 1.8µm column using a mobile phase composition of pH 6.8 buffer: Methanol: ACN (30:30:40) at a flow rate of 1.0 mL/min. UV detection was performed at 333 nm. The method was validated for specificity, linearity, precision, accuracy, robustness, ruggedness & solution stability according ICH guidelines. The calibration plot was linear over the concentration range 12.5-75ppm with correlation coefficient 0.9998. The accuracy was good. The HPLC method developed is accurate, precise, reproducible, specific, and stability indicating. The method is linear over a wide range, economical and utilizes a mobile phase which can be easily prepared. All these factors make this method suitable for quantification of Nisoldipine in bulk drugs and in pharmaceutical dosage forms without interference. It can therefore be concluded that use of the method can save much time and money and it can be used even in small laboratories with very high accuracy and precision.

DISSOLUTION

An isocratic **RP- HPLC** method for analysis of Nisoldipine in pharmaceutical dosage form has been developed and validated. Best separation was achieved on a **PRONTOSIL C_{18} H** (100 × 4.6 mm) 3µm column using a mobile phase of composition of pH 3buffer: ACN: Methanol (30:40:30) at a flow rate of 1.5mL/min. UV detection was performed at 333 nm. The method was validated for specificity, linearity, precision, accuracy, robustness, ruggedness & solution stability according ICH guidelines. The calibration plot was linear over the concentration range 10-60ppm with correlation coefficient 0.9997. The accuracy was good. . The data validation shows that the RP-HPLC method is accurate, robust and possesses excellent linearity and precision characteristics. This method can be successfully used for the quantitation of Nisoldipine as active substance, in dissolution studies and in tablet dosage forms. So it can be concluded that the developed method can be easily applied for the routine quality control analysis of Nisoldipine drug and it’s Formulation.

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